

Slamming the Wrong Stable Door?

LIKE cowboys reaching for their guns, politicians are increasingly tempted to respond to pressing and urgent problems by creating special agencies, government departments, task forces or other conspicuous instruments of public administration. Only a few weeks ago (see *Nature*, 231, 140; 1971) in Britain, the House of Commons Select Committee on Science and Technology was asking for a Special Office separate from other established branches of government to consider problems of population policy, and so far it has earned more kicks than ha'pence for its foolishness. Now, in the United States, there is a risk that President Nixon has taken a step down this primrose path by endowing with \$155 million of new money a Special Action Office for Drug Abuse Prevention, an instrument of government independent of other agencies but with responsibility for coordinating what happens elsewhere. President Nixon himself described his new programme last week as an "all-out offensive" on drug abuse, and nobody will quarrel with the good and even humane intentions which have inspired this dramatic step. Precisely because the problems are important, however, it is proper to ask whether the methods and even the objectives of what is now intended are those most likely to help ameliorate the abuse of drugs in the United States. The steps now proposed have the form of ways of making the symptoms go away without removing the underlying causes of drug abuse.

The scale on which drugs are being used in the United States is, without question, a source of great alarm. This is beyond dispute. Heroin addicts are thought to exceed 200,000, about a half of them in New York. Something like fifteen per cent of the United States troops in Indochina are thought to have used heroin at some stage or another. At home more than ten million people in the United States have probably used marihuana at some stage or another, although there is also some reason, mostly subjective, to think that the great middle-class preoccupation with marihuana is beginning to fade away; the commonness of more serious forms of addiction has probably overshadowed what must increasingly seem an effete behaviour.

But what is to be done? That, unhappily, is a more difficult question. To be sure, there should be more facilities for the treatment of heroin addicts, and since this is where the biggest slice of the extra money will be spent—an extra \$105 million—the new proposals will probably bring benefit to many people. Indeed, the chances are that there will now be a modest but deliberate expansion of the methadone programmes which have been introduced over the past few years on the basis of the work carried out in New York in the fifties and sixties by Dr Richard Dole, and pursued with particular flair in Chicago by Dr Jerome H. Jaffe, who will now become the director of Mr Nixon's Special Action Office. By now, after all, there is plenty of evidence that methadone therapy can be effective in favourable circumstances. The problem is that favourable circumstances are expensive both for the patient and for those who treat him—most patients must attend regularly at a clinic, while methadone treatment is beyond the competence of general practi-

tioners simply because of the range of services with which it should prudently be accompanied. And even so, there are dangers, not the least of which is that casual or inefficient treatment may make available to people in general, many of whom have never been addicted to heroin, supplies of a synthetic material which is, when all is said and done, addictive in its own right.

So far as it goes, this part of the programme is sensible enough, but the most obvious difficulty is that if there really are 200,000 heroin addicts in the United States, the \$200 million or so of federal funds now available each year for treatment and medical research is unlikely to meet the whole need. It is hard to see how it would be possible to spend more money quickly, but it would be as well for people to recognize at the outset that the strictly medical part of the programme now proposed cannot deal with the whole problem, only with a part of it.

Other parts of Mr Nixon's programme are more questionable. A great deal of talk and a good deal of money are to be spent on the enforcement of existing law. There are to be research programmes for the more efficient detection of smugglers of addictive drugs, the three bureaux of the Federal Government responsible for the enforcement of the law are to be strengthened and there is even to be a research programme in the Department of Agriculture to develop potentially selective herbicides that could be used for destroying fields of opium poppies without doing ecological damage to surrounding forms of life. It is not entirely clear whether the United States Administration has at the back of its mind that it might be possible to stamp out heroin manufacture by spraying Turkish poppy fields from the air, but the President's message last week was strong on the need to strengthen international action against the poppy growers. The Government of Turkey, quite properly, is in for some plain speaking.

The trouble in all this, of course, is that there is no assurance that the mere restriction of heroin supplies will restrict the recruitment to the ranks of the addicted. There is at least a chance that greater scarcity, even if it could be brought about by the stricter enforcement of domestic and international laws, would serve first of all to drive up the price and thus still further increase the difficulty which addicts already experience in paying for their fixes. To be sure, methadone is cheaper and can be used in therapy within the law, but in the circumstances which now obtain in the United States, is there not at least a case for asking whether there might be advantages in a state heroin monopoly, with supply restricted to registered and verified addicts at no cost worth speaking of and in circumstances in which it would be possible to monitor the symptoms of their condition and, occasionally, to wean them from the addiction? Dogmatically to advocate such a course would, of course, be foolhardy, but there is a strong case for asking whether an efficiently organized version of the system used until recently in Britain for the treatment of heroin addicts may have a part to play in the extraordinary circumstances now to be found in the United States. To be sure, much more would have to be known about the use of heroin in a



maintenance regime. Obviously there would be risks, not least of all because heroin seems to lack the self-regulating characteristics of methadone, so that external control of an individual's use of the drug is in some form essential. But there can be no doubt that the use of heroin in the treatment of heroin addiction would put the pushers to rout and would at the same time make it easier to extend the treatment of serious addiction to those among the heroin addicts who lack either the will or the wish to become dependent on methadone. The difficulty, of course, is that such a policy would entail a turnabout on policies towards addiction, but that is no reason for ignoring the issue. One of the most serious weaknesses of Mr Nixon's programme is that its fine intentions are not supported by a sufficiently reflective analysis of the problem to be solved.

What else might be attempted? It is a mere platitude to say that the most urgent need is a better understanding of the causes of drug addiction. Simply to know more about the ways in which people are first persuaded to try their luck with marihuana or heroin will not keep them safe. By now, after all, the causes of juvenile delinquency are well documented, but this does not keep young people out of trouble. To reap the benefit of what the social scientists have to say about drug addiction requires applied research in every way comparable to the

technical development necessary to make economic sense of engineering innovations. Unhappily, there is no very clear concept of how such work should be planned and organized. There is no doubt a good deal to be gained from the programmes with formally educational objectives now being supported generally with public funds in the United States, just as there may be in more adventurous schemes such as those in which community life of one kind or another is offered as a means of helping addicts do without their drugs. In these directions, however, it is clear that serious work is only just beginning. Last week's new programme does, it is true, include a further \$10 million for education. It is also clear that the programmes of research already mounted by organizations such as the National Institute of Mental Health will in due course provide valuable results. Yet the tone of Mr Nixon's statement last week, like the balance of the ingredients in his package of proposals for fresh expenditure, smacks too much of the familiar talk of law and order, and is too neglectful of the opportunities for constructive work on the causes of addiction. Unless those in charge are careful, there is even a danger that too much emphasis on action will obscure the problems which really need attention—those of providing a full and sensitive picture of the reasons why drug addiction has blossomed in the past few years.

Making Virtue of Necessity

EVIDENTLY this will be a trying summer for those on both sides of the English Channel who hope that some time in the foreseeable future the European Economic Community will be enlarged so as to include the offshore islands and peninsulas to the north. Since the beginning of the latest series of negotiations in Brussels, it has been clear that decisions about the enlargement of the community would hang on the question whether the United Kingdom would be satisfied with the terms offered by the existing members. As the weeks have gone by this year, it has become apparent that the chances of a satisfactory agreement are now high. The danger now is not so much that the negotiations in Brussels will fail but that, even if they succeed, the British Government will fail to carry enough political support to be able to sign up with the EEC.

In retrospect, it is strange to see how many problems have apparently just melted away. Ten years ago, for example, there were politicians and others in Britain prepared to go to the stake for the retention of sterling as an international reserve currency, with all the accompanying apparatus of sterling deposits (debts to foreign governments, usually ex-colonies or members of the British Commonwealth). Now, the interests of British governments lie almost at the opposite pole—a succession of financial crises in the sixties has persuaded even the chauvinists that British interests might be better served by a rational system of international monetary agreements. In exactly the same way, there is now much less protest, in France as well as Britain, that the mere existence of the EEC will in due course lead to political, not merely economic, association. So what? For is it not now apparent, in fields such as military defence, that Europe is already far too small for its separate parts to function independently of each other? And who these days hears

from either the British or the French about the need that they should separately or even jointly keep up with the Joneses in the development of nuclear weapons? The sixties have evidently helped many European nations to a better appreciation of reality.

It is thus ironic that the prospects of the enlargement of the EEC should be jeopardized not so much by the difficulty of coming to an agreement as by the difficulty of persuading the British electorate to appreciate which side their bread is buttered. It is not, after all, so very long since Britain was engulfed in indignation that the Government of France should say that Britain should not be in the EEC. It is especially ironic that the reasons which now apparently persuade the doubters in Britain that the EEC would be an intolerable risk are in themselves powerful demonstrations of the economic necessity of membership of the EEC. Yet the collapse last week of yet another large British company—Upper Clyde Shipbuilders—should be a reminder of how vulnerable are many traditional British industries in the tiny market on which they have now become dependent.

There is no magic in this apparently inexorable process of decline. Where shipbuilding is concerned, for example, it is most probable that if it had not been for the isolation of the European economies from each other in recent years, British shipyards would have long since migrated from the rivers on which they were established in the nineteenth century. Only the economic inflexibility of the recent past has now made coelocanth of them. But the lessons to be gleaned from shipbuilding could be multiplied in all kinds of British industries, from textiles to aircraft. To be sure, there are many who argue that if the economic advantages of the EEC are merely those of a customs union, and if the general level of tariff barriers is declining (as it is), then British membership

of the EEC should be commercially unimportant and should become even less so as the years go by. The flaw in this argument is that it overlooks the difficulties of making radical changes in the pattern of industry within a comparatively small country. Understandably, and sometimes with good reason, governments seek to resist the forces of industrial change when it seems that the consequences may include the running down of an important industry or even its disappearance. Even without tariff barriers, there are easily recognized ways in which governments can bolster up their domestic manufacturers. In Europe, one obvious result has been that the continent's productive capacity has not grown in the ways and at the places that would be most economic. Another is that similar and neighbouring European governments have so often followed similar policies that their efforts are often slavishly duplicated.

To the extent that the question of how to respond to the EEC requires that British electors should take a view about the development of industry in the next few decades, there are valuable lessons to be learned from recent industrial history. The most striking of all is the way in which, only a few years ago, Europe was preoccupied with what was then called the "technology gap"—the supposed disparity between the United States and the rest of the industrialized world in the capacity to exploit advanced technology. The outward symptoms of this disease were such things as the markedly inferior prosperity of Western Europe and the way in which European companies were apparently less capable than their counterparts in the United States of exploiting advanced technology, electronic computers, for example. It is highly significant that this obsession has not quite disappeared. European prosperity still leaves much to be desired, while European companies are still unsuccessful in their attempts to manufacture large workable computers. The chief difference seems to be that European industry has become very much more efficient than a few years ago at performing all kinds of conventional tasks, from the manufacture of steel to the farming of agricultural land. The result is that the people engaged in these enterprises have a pleasant sense of knowing that their lot is steadily improving, and this is eminently a field in which it is better to travel than to arrive. Moreover, there is now every prospect of further gains from the continuing improvement of the efficiency of European industry. Heroic innovation is plainly much less necessary than it used to seem. The moral in this for common marketeers, on or off the mainland, is that there is no intrinsic impediment to the continuing growth of European prosperity. The practical question is to know how to make the process as easy and as painless as possible.

Short though the time may be between now and the point at which the British Government will have to take its courage in both hands later in the year, there are a number of constructive steps which could be taken to persuade the doubters that there is useful work to do. One of the most important is to demonstrate explicitly the nature of the demands which the EEC is likely to make on countries such as the United Kingdom. A good deal of the implicit resistance to the idea of membership springs from fear that extraordinary feats of innovation will be needed if industry in the member nations is to remain competitive. To many people, it should be something of a reassurance that the most immediate need is

for more of the kind of patient attention to the techniques of management that British industry has been trying to teach itself for several years. There is, moreover, the further prospect that present arrangements in Britain for the improvement of industrial efficiency will turn out to be valuable assets in a European connexion. Diffident British taxpayers have only to reflect on the benefits which there may be from such things as the service laboratories which abound in the support of British industry—institutions for such things as the design of hydraulic works or the improvement of industrial standards—to know that they may quickly be able to play a useful (and profitable) part in the further development of the European economy.

But how are these benefits to be obtained? This is the point on which the British Government should concentrate in the months ahead. No harm would come of an attempt to show, in detail not in outline, just how the facilities which at present abound in Britain for the support of industry would be deployed if Britain (and its other northern associates) were to belong to the EEC. Would the organizations and research laboratories at present dedicated to the support of British industry be able to operate on a wider canvas? Evidently there is a strong case for some closer integration of the publicly supported industrial research organizations of the several European nations, and it is not too soon to know just how this would be accomplished. But there are also useful ways in which universities might be persuaded to collaborate on a European basis, and here again there is much to be said for working out in advance ways of pooling these common resources. No doubt, similar steps could be taken in other fields to show that European integration does not necessarily spell disaster. In short, the breathing space between now and the point at which the British Government will be in a position to decide on the European question can be fully and constructively occupied. Indeed, unless there is a serious attempt to anticipate as fully as possible the consequences of membership, the doubters are likely to gain too much in influence.

100 Years Ago



PARIS

Academie des Sciences, June 19.—M. Claude Bernard in the chair. M. Claude Bernard read a letter from Mr. Alexander Herschel, noticing the death of his father on behalf of himself and of his eldest brother now in India. The lamented Sir John Herschel was the senior foreign associate member of the Institute. The foreign associate members are only five in number; it is considered the highest honour the Academy can offer to a foreigner. The President noticed also the death of the celebrated General Probert, who was an academician of long standing, and had devoted his whole life to the study of projectiles. His memoirs are numerous in the *Comptes Rendus*, but more numerous at the War Office. He was of opinion that the Prussian steel gun should be adopted by the French artillery, but his Imperial Majesty being a great artillerist, his opinion was totally disregarded.

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OLD WORLD

BOX GIRDER BRIDGES

Under Surveillance

WHATEVER conclusions the engineers of the Department of the Environment reach about the 42 box girder bridges in Britain whose safety seems in doubt, the recent disasters involving bridges of this type in Wales and Australia show that there is some way to go before these structures are fully understood.

Box girder bridges are commonly of two types; a single, flat steel tube of rectangular or trapezoidal cross section which might be 70 feet in width and 10 feet deep, or a pair of smaller tubes measuring only 10 to 20 feet in width. The concrete roadway is laid over this basic support structure. Lengths of box girder, which are simply four steel plates welded together to make up the appropriate shape, are easily manufactured, and they are attached to each other *in situ* to make up the whole span.

Box girders have become important in bridge building because they require less steel than a conventional I-girder structure; they are also simple to maintain and present a clean aerodynamic profile. Furthermore, the torsional stability of box girders, in comparison with I-girders, means that load distribution is more efficient. In some conventional bridges, on the other hand, the design must allow for a considerable proportion of the load to be supported on one girder, a measure which considerably increases the amount of steel which must be used. Curved elevated roadways are in torsion even when the loading is symmetrical, and box girders offer definite structural advantages for such bridges. Lastly, the slim lines of a box girder bridge are more aesthetic (and therefore appeal more strongly to an engineer's design conscience) than those of a conventional I-girder bridge.

Despite the appearance of simplicity, the analysis of the behaviour of box girders under load is complex. The behaviour of a long plate of steel firmly bonded at the edges but in a state of compression is especially intractable; the top surface of a box girder, welded to the side plates at its edges, presents just such a problem. Compression causes an undulating distortion—buckling—which has to be calculated and taken into account in the overall design of the bridge. One of the ways of counteracting the tendency to buckle—which depends on the square of the ratio of the effective plate width to the thickness—is to weld several stiffeners along the length of each plate so that the width of plate between the stiffeners is quite small. And, of course, none of this allows for the inevitable locked-in stresses which occur during welding.

The critical parts of a box girder bridge, from the design point of view, are the regions close to the centre of a span, and those around the piers which support the girders. The arched shape of a typical span means inevitably that the top girder surface near the centre of the span is in a state of compression, but this stress is rather more evenly distributed when the concrete roadway is bonded to it. What must be taken into account, however, is that there must be a stage in construction when the concrete is setting and therefore not adding strength but merely deadweight to the structure. The second important feature is the design of the portion of the box girder around each pier support. Because of the normal reaction at the support, extra reinforcement is necessary. A steel diaphragm is welded to the inside of the box and this is reinforced again with a stiffening plate immediately above the support itself.

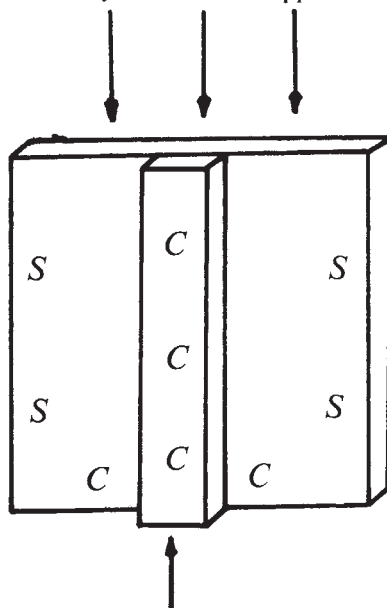


Diagram of a stiffened diaphragm (not to scale). Arrows indicate the forces acting. C, Regions of compression; S, Regions of high shear stress.

The diagram indicates the patterns of stress in the diaphragm; in the areas marked C, the diaphragm is in a state of compression, but at S the material is in a state of high shear stress which is, in turn, transmitted to the sides of the box. The evidence suggests that it was failure of the diaphragm that was responsible for the collapse of the Milford Haven bridge during construction and it seems likely that if modifications to box girder bridges are necessary in the interests of safety it will be the diaphragms and the sides of the girder in their immediate vicinity which will be strengthened.

But modifications like these mean fresh headaches for bridge builders; the true answer is to find new ways to overcome the problems in designing and manufacturing effective box girders.

TELECOMMUNICATIONS

Post Office Goes Dutch

TWO years ago, the British telecommunications industry was shaken when the Greater London Council gave the contract for its new internal telephone system to the Swedish firm of L. M. Ericsson. Now it has seen an even more valuable contract—for an electronic telegram switching system—go to another foreign competitor, Philips of Eindhoven. The system, worth £3¼ million, may be the largest message-switching unit in the world. It will automatically collect, store and forward to the proper address 27,000 telegrams an hour, not only to destinations in Great Britain but overseas.

Why Philips? The Dutch-owned company, represented in this contract by its British arm, the Telephone Manufacturing Company of Dulwich, seems to have impressed the Post Office with its blend of expertise with computers and experience with conventional telecommunications message switching (some of which is now used by the Post Office for its international telegraph traffic). Computer manufacturers themselves have not so far been very successful in producing electronic telephone exchanges for use in public networks. Telephone suppliers, on the other hand, tend to be unsophisticated in computer technology. One of the British contenders for the contract—Data Communication Sciences Limited—is a company formed specifically in order to bridge the computer-telecommunication technology gap. It is owned by International Computers and Plessey Limited and the loss of the contract must be a disappointment to both.

The telegram switching system will be installed at the Post Office's Cardinal House in London. It will be based on a DS 714 telegraph computer and will replace the present electromagnetic and torn-tape methods of switching telegrams. In the former, a human telegraph operator reads the address on an incoming teletyped message, then re-types it in full to send it to its destination. The torn-tape method is an improvement in that the operator does not have to retype the message but simply to tear off the incoming perforated tape and feed it into another machine for retransmission. The computerized system will perform the whole process automatically, holding back low-rate and non-priority messages until off-peak hours.

If the contract is an achievement for Philips, so it is for the Post Office as well. The corporation has not been accustomed to commit itself to comprehensive operations of this magnitude and it may gain confidence to engage in similar boldness in the future.

NEW WORLD

Conflicting Philosophies over 2,4,5-T

by our Washington Correspondent

A SCIENTIFIC committee appointed by the administrator of the Environmental Protection Agency, William D. Ruckelshaus, has recommended that all existing restrictions on the use of the herbicide, 2,4,5-T be lifted. A strongly dissenting minority report written by one committee member is one reason why Ruckelshaus has a vexed decision before him. Another is that the subject matter of the report raises wider issues than that of the use of 2,4,5-T, such as the competence of scientific committees to assess the risks the public can be asked to accept, the frame of mind in which such a committee approaches its task, and the question of how far the data by which these risks are estimated should be made public.

The report of the committee itself, for instance, is not intended to be made public until after Ruckelshaus has announced his decision on 2,4,5-T, if then. A copy of the report, however, has been made available to *Nature*, from which it is evident that a crucial issue divided the committee, namely the matter of whether the additional research required to establish the safety of 2,4,5-T so far as it can be proved should be carried out before or after re-exposing the public to the herbicide. The majority of the 10-member committee took what may be described as the conservative view, that the public could be exposed first and the experiments done afterwards. Only one member took the opposite view, holding that, slight as the risks may be, the additional research should be done first. Both sides justify their positions on the grounds of more general considerations. James G. Wilson, professor of research paediatrics and anatomy at the University of Cincinnati and chairman of the committee, believes that since all chemicals that find their way into the environment are potentially toxic, the foremost job of a scientific committee is to establish the quantities of the chemical that can be tolerated. Theodor D. Sterling, professor of applied mathematics at Washington University, St Louis, and author of the minority report, argues that the old attitude towards balancing economic interests against public health has been outmoded by recent experience with compounds such as DDT and mercury and their unexpected growth and burden in food chains. It falls to Ruckelshaus to judge this issue on the basis of the committee's report and

whatever other evidence he may be able to draw on.

The herbicide 2,4,5-T first came to public attention during its peak years of use as a defoliant in Vietnam; for a time the military demand was so great that the herbicide almost disappeared from the domestic market, where it was used chiefly in land and waterway management, and to a lesser extent on crops. Although 2,4,5-T has been on the market since 1948, its teratogenicity passed unnoticed for almost twenty years. Experiments conducted by the Bionetics Research Laboratories under contract to the National Cancer Institute first cast doubt on the safety of the herbicide. These experiments, completed in about August 1968 but not made public until October 1969, indicated that low doses of 2,4,5-T given to pregnant mice frequently caused malformations in the offspring. It was not until April 1970 that the Surgeon-General announced that 2,4,5-T would be suspended for use over water, around the home and on food crops, thereby minimizing the risk of exposure to pregnant women. The Department of Defense announced at the same time that it would observe these restrictions in the military use of 2,4,5-T.

Two of the manufacturers of 2,4,5-T, Dow Chemical Company and Hercules Incorporated, exercised their right under the Federal Insecticide, Fungicide and Rodenticide act to petition for an advisory committee to re-examine the need for the restrictions, and the report now before the Environmental Protection Agency is the result of this committee's work. The majority recommendations of the committee are as follows: 1. All restrictions on the herbicide should be lifted. 2. The use of the herbicide should be governed so that residues of not more than 0.1 part per million remains in food in drinking water. 3. Manufacturers must limit to 0.1 ppm the contamination of 2,4,5-T with dioxin (the highly lethal by-product of 2,4,5-T manufacture that confused interpretation of the original Bionetics experiments), but existing stocks of 2,4,5-T may be sold as long as their dioxin content does not exceed 0.5 ppm. (For use around the home, however, existing stocks of 2,4,5-T must not contain more than 0.1 ppm of dioxin.) 4. Formulations for use around the home must carry a label warning that the herbicide may be dangerous to preg-

nant women and animals. 5. Further research should be undertaken (the committee does not say by whom) to study whether dioxin accumulates in the soil and in food chains, with these issues being examined again in three years' time. 6. Agricultural chemicals should continue to be tested for untoward effects even after they have been registered for use.

The minority report submitted by Theodor D. Sterling recommends that existing restrictions on the use of 2,4,5-T be maintained but that the herbicide be permitted for use in forestry and maintaining rights-of-way subject to certain conditions. These are (i) that the dioxin content be limited to 0.1 ppm, (ii) that 2,4,5-T be applied no more than once a year to the same site, and (iii) that the herbicide be applied with proper care to ensure it does not come into human contact.

"The fact that ours is the view of a minority," Sterling says, "ought to strengthen the impression that the committee laboured honestly and conscientiously to deduce the best recommendations from a confused aggregate of observations." There is no doubt about the committee's probity and devotion to duty—one member estimates that he spent 150 hours exclusive of the three committee meetings in studying the 1,700 pages of material. And the chairman of the committee took the unusually meticulous step of performing in his own laboratory and without financial assistance a particular study of 2,4,5-T that had not been done and he felt ought to be. Sterling and his colleagues nevertheless disagree both on matters of substance and attitude.

Perhaps the most serious point of contention concerns the hazards of the dioxin contaminant. Dioxin, as the committee points out, has an LD₅₀ of 0.0006 mg/kg in the guinea-pig (in other words will kill half of a population exposed to that dose) and when fed to pregnant rats causes embryotoxicity at doses of 0.000125 mg/kg. As such it is one of the most toxic substances known. The EPA committee concludes that although dioxin "has been shown to have a low teratogenic potential at doses in excess of 0.001 mg/kg", it is "virtually impossible" for anyone to be exposed to this dosage with currently produced 2,4,5-T. This conclusion is based on the belief that dioxin does not accumulate in air, water or plants (although its ability to accumulate in soils has yet to be resolved), and that,

on the basis of a partitioning experiment between water and fat, dioxin is so insoluble in fat that "it would not be expected to accumulate in body fat depots in appreciable amounts".

Sterling objects to the committee's views on dioxin on two accounts. First, "the data do not necessarily justify a conclusion that there is a level at which TCDD [dioxin] is not teratogenic" and, second, "the report fails to consider the consequences of the (admitted) uncertainty about the fate of TCDD in the food chain and in tissue". The report gives the impression, Sterling says in his minority report, that 2,4,5-T is only teratogenic at high doses, but the dose effect depends largely on the amount of dioxin impurity it contains. The experiments and analysis on which the committee have had to rely "unfortunately were not done with the sophistication necessary to throw light on the effect of 2,4,5-T and dioxin at very low doses". Sterling states that there are nevertheless sufficient instances where teratogenic properties have been observed at doses lower than 100 mg/kg of 2,4,5-T (most studies set 100 mg/kg of 2,4,5-T as the lowest dose in the range, so that there are few data about the teratogenicity of 2,4,5-T below this dose). As a case in point, Sterling cites the experiments specially undertaken for the report by the committee chairman, James G. Wilson. These experiments, undertaken to study the effect of a single treatment of 2,4,5-T on rat foetuses during the period of organogenesis, provided for doses of 100, 200 and 400 mg/kg but only tests for a single low dose, that of 20 mg/kg, although "the effect of a dose at this low level is of the utmost importance". Sterling also charges that the experiment is without controls. The controls cited in the report refer to cumulative experience with animals used over the past four years. "Given our knowledge of variation in experiments, it is difficult to understand why this important experiment was performed without a concurrent control." The experiment nevertheless shows a trend towards lower dose which is still detectable at 20 mg/kg. In view of these considerations, Sterling says, it "becomes difficult to see how the report can conclude that 'doses of low-dioxin-content 2,4,5-T and of TCDD [dioxin] below the level producing maternal toxicity were without significant effect on prenatal development [in various species]. . . .' If anything, the conclusion ought to have read that, despite the small amount of data present, some of it does point to teratogenicity at lower doses."

Sterling said last week he found it "incredible" that proper controls were not used in the Wilson experiments. Asked whether he had raised the question with Wilson, Sterling said he had

not, because "you don't tell a man of his age and experience how to conduct experiments". Wilson commented last week that the use of fully concurrent controls would have made no difference to the conclusions drawn from the experiments and that some of the animals cited as cumulative controls were in fact concurrent controls. Asked whether the separation of the concurrent controls from the cumulative would have made any difference to the data, Wilson said he stood by his experiments. Another member of the committee defended Wilson's experiments on the grounds that they were "done in a very short time without financial resources" and that "Wilson would never have published these data". But data that are not fit to be published in an academic journal are hardly any more fit to serve as the basis for a decision affecting public health. Asked last week if he planned to publish his experiments, Wilson replied that he had not yet considered the matter.

Another point at which Sterling finds the committee too sanguine over dioxin is in the estimates of what happens to the compound in the environment. Although the report notes that dioxin may accumulate in the soil, Sterling points out that "there is no information on the concentration of dioxin in the food chain at [the level of plants], nor is there information as to what extent TCDD may be stored in animal tissues. . . . The report speculates that TCDD is not stored to a significant extent because it is not easily soluble in oils. However, may it not be stored by other tissues besides fat? . . . After recent experience with DDT and mercury, it would be reckless to leave such questions in abeyance while approving the unrestricted use of 2,4,5-T."

An important body of data the committee tried to assess was studies of the effects of crop destruction and defoliation programmes in Vietnam which were undertaken by the US Army and by the Herbicide Assessment Commission of the American Association for the Advancement of Science. The Army study concluded that no human birth defects in Vietnam were attributable to herbicide spraying and the AAAS commission decided that the birth data it studied were indicative of a possible link between herbicides and defects but that the data were not sufficient to draw any firm aetiological conclusion (see *Nature*, 229, 223; 1971). The EPA committee devotes several pages of its report to describing the unreliability of the data with which the AAAS study worked and concludes with the statement, quite remarkable for a scientific body, that not only is the relationship between herbicides and birth defects in Vietnam unknown, it is also unknowable. "Any attempt to re-

late birth defects or stillbirths to herbicide exposure is predestined to failure," the committee declares. Told last week of this conclusion, Matthew S. Meselson, biology professor at Harvard University and designer of the AAAS study, said he found it extraordinary and outlined how a survey, based on interviewing women about their birth history and residence in sprayed or non-sprayed areas, could be conducted so as to avoid the difficulties enumerated by the EPA committee.

The committee's declaration that all studies of the relationship between herbicide spraying and birth defects are predestined to failure presumably extends to the study of the physiological effects of defoliation in Vietnam now being undertaken by the National Academy of Sciences for the Department of Defense. The study was commissioned by the department at the express request of the Senate.

One member said last week that the committee's comment on the birth defect/herbicide studies being predestined to failure was "an overdramatic and rather poor way of putting it". Sterling in his minority report claims the report evinces an "unjustified certainty" that Vietnam birth records do not show teratogenic effects. The finding by the AAAS of increased stillbirth rates in certain areas may, it is true, have been a spurious effect, unrelated to herbicide spraying, but the opposite could just as well be true. "Factors could just as easily have worked to hide a large stillbirth rate than to spuriously create one," Sterling says. "If we already speculate, we might just as easily speculate the other way. Thus, the best we can say is that these data do not definitely show an effect of defoliation. However, they do show an effect which has to be explained away. It is up to the committee to register our doubts, but it is unseemly to spend page after page denying the reality of the Vietnam observation in the face of the careful report by a select committee of the AAAS."

Another failing cited by Sterling in the minority report is that the committee "does not weigh risk versus benefit, as it was charged to do". Sterling's own views on the benefit of 2,4,5-T are that although the herbicide is of major value in its non-farm uses, such as forestry and road clearance, it is less useful around the home and on food crops, yet the latter two uses expose the largest numbers of people to 2,4,5-T and its impurities. "Thus it is not true that all uses of 2,4,5-T are equivalently beneficial." Sterling concedes that the non-farm uses of 2,4,5-T clearly have national benefit but the use of the herbicide for growing crops, especially rice, "is basically of benefit to a few farm

industries because it increases the yield per acre of cultivated ground. Since there are available many acres that are not now cultivated, there does not appear to be a national urgency to support that limited use of 2,4,5-T until important questions about the build-up of 2,4,5-T have been settled."

Risk versus benefit calculations are not the only omission from the committee's report. Another matter on which the report is less than explicit is a faulty experiment undertaken by the Bionetics Research Laboratories. The presentation the committee heard from one of the petitioners, the Hercules Corporation, was based on what was supposedly a repeat study of one of the original Bionetics experiments indicating that 2,4,5-T was teratogenic. In the original study, two strains of mice were given a 113 mg/kg dose of 2,4,5-T and a high percentage of abnormalities was found in their offspring (see *Science*, 168, 864; 1970). Since the 2,4,5-T used for the experiment was later found to contain about 30 ppm of dioxin, the experiment was repeated by the Public Health Service using 2,4,5-T formulations containing less than 0.5 ppm of dioxin but the same birth defects were observed. The Hercules Corporation asked Bionetics to repeat the Public Health Service study and reported to the committee that Bionetics had been unable to confirm the PHS finding of teratogenicity. The committee sent the new Bionetics study out for review to K. Diane Courtney, a member of the National Institute of Environmental Health Sciences, who undertook the original study for Bionetics. Courtney pointed out that the repeat study of 2,4,5-T had used the wrong dosage of 2,4,5-T, 10 mg/kg instead of 113 mg/kg. Bionetics repeated the repeat experiment with the correct dosage and found that the purified 2,4,5-T caused cleft palates in the mouse embryos with a frequency of 11 per cent or 1 per cent depending respectively on whether Dow's brand of 2,4,5-T or that made by Hercules was used. Curiously, the EPA committee makes no mention of Bionetics' first attempt at the experiment, which Courtney describes as due to an "honest mistake with the decimal point". Asked why the Bionetics error was not mentioned in the committee's report, chairman Wilson said that he had "forgotten it wasn't", adding that he would "just as soon not air it" and that "they made a mistake—so do we all".

Other differences between the majority and minority parts of the committee include the question of the attitude the committee took towards its inquiry. Certain passages in the report suggest that the committee was unduly conservative in its concern to

take the industry position into account. "The report presumes to lecture the scientific community on the wisdom of instituting a 'permissible residue' of substances thought to be teratogenic," Sterling observes in his minority statement. Another indication of a perhaps conservative attitude is the committee's refusal to take personal evidence from the Environmental Defense Fund. "I feel the committee did not pay sufficient attention to the point of view of environmentalists and ecologists," Sterling said last week. "We had debate and discussion about this, and the majority of the commission wanted to do this as a courtesy, since we were hearing from the petitioners. But the chairman wrote to them saying it was not necessary to hear them since they had no new data."

Wilson and two other members of the committee said last week that there was not a majority in favour of hearing evidence from the environmentalists, besides which, Wilson added, Sterling voiced no objection to the agreed procedure at the committee's meetings. Sterling, however, says that at the committee's first meeting the "general opinion was that we ought to give time to the environmentalists, especially since we were going to hear industry. At the second meeting Wilson informed us he had already written a letter to the environmentalists saying that the committee would not meet with them. It was a *fait accompli* and no one was going to make a big thing out of it."

Wilson said last week that he had been asked by Ruckelshaus not to discuss the substance of the report until the administrator had announced his decision. Wilson was thus prevented from answering the specific criticisms of the main committee report made by Sterling. He drew attention, however, to the 9-1 verdict of the committee—"a more united decision than many of those reached by the Supreme Court"—and the scientific qualifications of the committee members. But although these are doubtless impeccable, the matter the committee was asked to resolve can only be helped by scientific expertise, impeccable or otherwise, in as far as it relates to assessing the empirical data about 2,4,5-T and its impurities. The crucial issue, that of weighing the risks of 2,4,5-T usage to public health against the economic benefits, is one that scientists are no better equipped to tackle than anyone else, which may be one reason why the EPA committee skirted round the question. And in so far as this aspect of the question is not scientific, otherwise extraneous considerations such as the attitudes of committee members towards industry and the environmentalists become of definite significance. The committee's perhaps understandable

failure to resolve the difference between the two conflicting philosophies represented on it has made all the harder the task of administrator Ruckelshaus in arriving at a decision on whether to lift or maintain the existing restrictions on 2,4,5-T. His decision is due 90 days after receiving the committee's report, which was submitted on May 7. In either event, the content and handling of the committee's report show how much of scientific basis on which the decision depends is concealed from public view until after the decision has been taken. The committee's report will not be released until after Ruckelshaus pronounces and maybe not even then. The members of the committee have been told not to discuss in public the substance of the report. The important experiments undertaken by the chairman of the committee may never be published and may thus escape the scrutiny of the scientific community. The committee neglected to inform the administrator in its report of the error made by the Bionetics Research Laboratory, and these experiments, too, may never see the light of day. By contrast, an academic monograph of no conceivable relevance to the public weal has to pass before several critical reviewers before being accepted for publication and once published is open to public criticism from the scientific community. From this process there is no exemption, it seems, excepting only for such issues as bear directly on the public health. The trouble with such exemptions, however convenient they may be to administrators in a hurry, is that, as Theodor Sterling said last week, "The consequences of us being wrong on 2,4,5-T would have severe repercussions for the whole basis of scientific advice in the matter of public policy."

SCIENTOLOGISTS

FDA Out for a Church

by our Washington Correspondent

IN a continuing misapplication of its limited resources, the Food and Drug Administration began this month a second attempt to get court approval of its eight year battle with the scientologist church. The matter of contention is 100 "E-meters" (galvanometers used to measure skin resistance) and some two tons of scientologist literature seized in 1963 by a posse of FDA officials and some Baltimore dockers impressed for the occasion. What business of the FDA's is scientology? The agency is responsible for checking the claims made on the labels of medical devices. In its suit with the scientologists, the FDA alleges that the E-meters are a medical device and that in the literature of the scientologist church—which the FDA says is the "label" for the device

—fraudulent claims are made for healing powers.

In February 1969 a Court of Appeals threw out an earlier verdict against the scientologists on the grounds that the jury had no business to pass judgment on the validity of religious literature. Now the FDA is trying again. Although scientologists should doubtless be regulated by some government agency, perhaps even the FDA, the FDA's record of prosecutions against quacks, frauds, and other easy targets which are not a part of the powerful food and drug industry is anything but honourable. For example, the agency conducted a thirteen year battle against Dr Wilhelm Reich, an otherwise reputable psychoanalyst who claimed that his "orgone box" could cure cancer and other diseases. Not content with prohibiting sale of the orgone box as well as of Reich's works, one of which is a classic of psychoanalysis, the FDA in 1956 also sent an inspector to visit Reich's house and supervise the burning of his books, an experience that Reich happened to have suffered before in Nazi Germany. (Reich was sent to gaol for refusing to obey certain provisions of the FDA's injunction against him, and died there a year later.) This shameful episode was condemned by the American Civil Liberties Union, but the FDA has not changed its attitudes. "If Dr Reich were alive today and published the same books, the courts would burn them again," an FDA official said last week. This threat will not of course deprive the food and drug industry of any sleep, but scientologists and their like stand warned of the FDA's consuming zeal for rooting out all manner of fraud, as long as it is not pharmaceutical or comestible.

UNIVERSITIES

Entrepreneurs Win Out

by our Washington Correspondent

WEAPONS makers are not the only people in the business of bidding for government contracts—universities also maintain and have thrived on an entrepreneurship relation with government. Last year the federal government support of universities dropped for the first time since 1963, totalling \$3,227 million or seven per cent less than in 1969. But the Massachusetts Institute of Technology nevertheless managed to increase its share of the largesse, becoming the first academic institution to receive more than \$100 million of federal government monies in a single year.

An analysis compiled by the National Science Foundation* indicates that a

major share of the cut was received by academic science, which between 1969 and 1970 declined by \$193 million or eight per cent, whilst non-science support dropped by \$33 million or three per cent. Of the six federal agencies that account for the bulk of federal support of universities, the largest spender is the Department of Health, Education and Welfare, which in fiscal year 1970 pumped more than \$2,000 million into the higher education system.

Of the other five main funding agencies, the National Science Foundation and the Department of Agriculture increased their support of universities by \$20 and \$26 million respectively between 1969 and 1970. NASA did better by \$4 million, but the Department of Defense and the Atomic Energy Commission did worse (by \$13 million and \$7 million respectively), the former in large part because of the Mansfield amendment requiring that research supported by the department bear a direct and apparent relationship to military need.

SCIENCE ADMINISTRATION

NSF Staff Out of Touch?

by our Washington Correspondent

By encouraging administrative rather than scientific skills, the National Science Foundation lets its professional staff members grow out of touch with their field of science. So states an internal memorandum composed at the request of the NSF deputy director Raymond Bisplinghoff. The memorandum also alleges that NSF staff members are given too little discretion, are poorly informed about administrative developments affecting them, and are discouraged from participating in outside scientific activities.

The author of the memorandum, Richard H. Hall, is a sociologist on leave from the University of Minnesota whose one-year secondment to the NSF ended last week. Hall worked in the NSF's institutional grants programme and, when that was cut back, as manager of a programme known as "Social System and Human Resources" which is part of a major NSF endeavour called RANN (acronym for research applied to national needs). Hall's specialty as a sociologist is the organization of professions.

The chief criticism in Hall's memo is that no effort is made to encourage the NSF staff member to "remain as intellectually alive as possible through continued participation in his own research . . . and active participation in his professional societies". Instead, there is an "overconcentration on administrative skills as an advancement criterion" which "becomes highly dysfunctional when decision about the

quality of the scientific endeavour must be made". As an example of this discouragement the memo quotes a director of the RANN programme as saying he "wants very much to do away with NSF staff members' participation in their own professional life through research and other activities".

The isolation of NSF staff members from their field of science, Hall said last week, has two chief disadvantages—first, that the scientist turned administrator tends to drop out of contact with the "invisible colleges" or informal communications network between members of his own specialty and, second, that there is a leaning towards relying on rules rather than an individual's own judgment. For example, in assessing an application for a grant, an administrator would be more concerned with whether the applicant was eligible rather than whether his idea was good. Asked for specific examples, Hall cited only a pending case in which he believed a good application risked being turned down because of doubtful eligibility.

Other criticisms made in the memorandum to Bisplinghoff include charges of too much supervision and poor internal communication. "The professionals are given much less discretion than their training would suggest is appropriate and the hierarchy does little in the way of communication, but sometimes too much in the way of supervision. This problem is compounded by the . . . tendency to promote sheerly on the basis of administrative skills or even sheer seniority so that the situation arises wherein the supervisors have no knowledge of what they are supervising." In the Social System and Human Resources programme, the memo continues, "the social science professionals were told what to do after having no input into the decision-making process about what was to be done."

Enlarging on this theme in an interview last week, Hall stated that social scientists had too little say in the formulation of the RANN programme. There is no social scientist among the triumvirate that directs the programme and little input from the social science research division of the NSF. "In both the institutional programmes and RANN the social science research division is less than cooperative," Hall said. Although he had been "disillusioned" by his year in the foundation he believes the NSF is "a good organization, probably as good as you could get". What notice had been taken of his memorandum to the deputy director? None, except that one of the aides to NSF director William McElroy, to whom a copy of the memo was sent, told Hall, "We must talk about this some time."

* *Science Resources Studies Highlights*, June 11, 1971. National Science Foundation, Washington DC 20550.

NEWS AND VIEWS

A Worldwide Marker for the Precambrian ?

HOWEVER many volcanic rocks come back from space, no astronaut is likely to bring back a piece of extra-terrestrial coal. The sedimentary rocks of the Earth reflect to such a remarkable degree the physical conditions at the planet's surface, and the nature of any biological activity which these have permitted, that these rocks are unlikely to be matched elsewhere.

On page 498 of this issue of *Nature*, Dunn, Thomson and Rankama discuss the rocks which formed during the great ice age which affected the Earth some 700–600 million years ago. They are particularly concerned with the possible use of these deposits as a worldwide marker with which to establish a subdivision of the late Precambrian. In putting forward this proposition they are elaborating a proposal made by Harland and other geologists from the northern hemisphere and are taking advantage of the particularly good conditions which Australia provides for the study of glacial deposits of this age. It has required 100 years to establish present knowledge of this worldwide event, for it was at the British Association meeting in 1871 that another Thomson suggested for the first time that certain boulder beds in Scotland now known to be Precambrian had been produced in a former ice age.

There are several remarkable things about these late Precambrian deposits. They are very widespread and reached the late Precambrian equator, which indeed ran close to the Scottish locality where the earlier Thomson made his discovery. They evidently date from a time when an exceptionally large area of continental crust lay below the sea, for they usually occur in marine successions overlying continental crust. A puzzling feature is the frequency with which dolomites occur in close proximity to the glacial beds which seem to have been deposited by drifting ice or possibly by grounded ice sheets.

If grounded ice sheets really reached the equator then the late Precambrian ice age must have been quite exceptional, as Spencer has emphasized (*Mem. Geol. Soc. Lond.*, **6**; 1971). It certainly covered a long period, though this may have been a time of successive glaciations separated by intervals when the climate was warmer. Dunn and his colleagues suggest an overall duration of 100 million years for the Australian examples. This is more than twice as long as the late Palaeozoic glaciation which reached a peak some 300 million years ago and about ten times as long as the most recent ice age.

Broadly speaking, three methods of measuring geological time are in use at present—the classical scheme making use of biological evolution; the determination of age through measurement of the decay of unstable isotopes; and schemes which are based on changes in the Earth's behaviour. Possibly the most promising of the third is the use of the periodic reversals of the Earth's magnetic field to establish a time scale which now covers the past 70 million years of geological time and which should in the future be extended further back in the history of the Earth. It remains to be seen whether climatic changes will provide a useful marker of time. Whether or not a worldwide event such as the accumulation of these late

Precambrian glacial sediments can be used as Dunn and his colleagues suggest, there can be no doubt at all as to the value of investigations which treat sediments on a global scale.

Very soon after the study of sediments began, it was realized that they could provide information both about past climates and about the former relief of the Earth. At first such reconstructions had to be tentative. As experimental work such as that of Bagnold on the movement of wind-borne sand, and of McKee on water-borne deposits, gradually established the physical conditions which could produce the features characteristic of different types of deposits, so the nature of the environments in which they might have formed became clearer. Equally important have been the studies of Recent deposits such as those of Shearman and Evans in the Persian Gulf which led to a radical reappraisal of the conditions under which evaporites of arid zones formed. A great step forward occurred in the 1950s when for the first time palaeomagnetic measurements provided an independent check of past latitudes deduced from geological evidence. In general, the geological reconstructions based on interpretations of past climates came through these tests with flying colours, and it was this agreement which showed beyond doubt that both methods could produce reliable answers. Armed with these results and with the ability to identify periods of break-up and of unusually rapid movement of continental masses it is now possible to make renewed progress in understanding past climates.

The study of sedimentary rocks on a global scale has taken several forms. In two recent issues of *Nature*, Gregor (*Nature*, **228**, 273; 1970) and Garrels and MacKenzie (*Nature*, **231**, 382; 1971) discussed the quantity of sediments formed annually and the length of time these are likely to survive before they in turn are destroyed by erosion. Exercises such as these can bring out the global relationship between the varying tectonic behaviour of the Earth and sedimentary processes. Another relationship emerged when it was discovered that, broadly speaking, the abundance of different types of sedimentary rock varies with palaeolatitude. It also has slowly become apparent that certain lithologies became particularly widespread in certain geological systems. As Robinson has recently put it (*Palaeontology*, **14**, 131; 1971), one might almost lose faith in uniformitarianism. As she points out, however, what can be seen here is a complex situation in which the degree to which continents are emergent or submerged, their overall size and their position relative to the poles must all be borne in mind. Here one is at grips with the interrelationships of movements affecting the outer parts of the solid Earth and changes in the circulation of the oceans and the atmosphere. Solving such problems may take a long time; what may be looked forward to now is a series of synoptic studies of climatic conditions over individual continents or the globe as a whole, on the lines of Robinson's review of the Trias or the work of Crowell and Frakes (*J. Geol. Soc. Austral.*, **17** (2), 115; 1971) on the Palaeozoic glaciation of Australia.

Regulating the Expression of Malignancy

MALIGNANCY is a heritable state; cells transformed by chemicals or by tumour viruses, for example, retain their malignancy during prolonged passage in culture and, in general, there is a satisfying correlation between the ability of cultivated transformed cells to induce tumours when inoculated into susceptible animals and their lack of growth control exhibited under a variety of tissue culture regimens. Both a cell's loss of growth control *in vitro* and its malignancy *in vivo* can, however, be reversed; by applying selection pressures to cultures of transformed malignant cells it is possible to select so-called revertants—cells which behave in culture as if they were normal, even though in the case of viral transformation the revertants retain the tumour virus genome. Such revertants, which in tissue culture exhibit growth control comparable to that of normal cells, are usually found to be no more malignant than untransformed cells when they are inoculated into susceptible animals. It is obvious therefore that the expression of transformation and malignancy can be modulated or suppressed. A cell which has a tumour virus genome(s) stably associated with and probably integrated in one of its chromosomes, or some other oncogene, may yet have the phenotype of a normal, untransformed cell.

Several research groups have reported in *Nature* and elsewhere experiments, all of which indicate that malignancy can be suppressed by changing the chromosomal constitution of a cell. Harris and his colleagues, for example (*Nature*, **223**, 368; 1969), suppressed the malignancy of a line of mouse cells by fusing them to non-malignant mouse cells; Pollack and his collaborators (*Nature*, **228**, 938; 1970) observed that when mouse 3T3 cells transformed by SV40 revert to a normal phenotype their chromosome number increases from a median value of 75 to 118. Likewise Rabinowitz and Sachs (*Nature*, **225**, 136; 1970) correlated the reversion of hamster embryo cells transformed by polyoma virus with an increase in the number of chromosomes. From this they postulated that the balance between two sorts of chromosomes—those carrying factors for the expression and those with factors for the suppression of transforming genes—determines whether or not the malignant phenotype is expressed. On page 511 of this issue of *Nature*, Hitotsumachi, Rabinowitz and Sachs describe new experiments designed to validate this suggestion.

They have now examined the chromosome complements of twenty-eight revertants of hamster embryo cells transformed by polyoma virus and find that reversion can be associated with a decrease as well as an increase in the chromosome number. Furthermore, reversion is not an all or nothing phenomenon, for the growth properties *in vitro* and the ability to induce tumours of revertants may fall anywhere in the spectrum between normal and malignant cells. That is not, of course, surprising if it is the balance between two sets of chromosomes carrying opposing factors that controls the expression of transforming genes. But if this hypothesis is correct there should be differences between the sorts of chromosomes as well as the number of chromosomes in normal, transformed and revertant cells.

Using the size, shape and position of the centromere as indices, Hitotsumachi *et al.* divided the chromosomes

of their revertants into a series of groups and compared these groups with their counterparts in normal and malignant cells. They believe that they have found that reversion, a decrease in the expression of the transformed cell phenotype, is correlated with a decrease in the number of chromosomes in one particular group, group 5. Chromosomes of this group therefore probably carry factors stimulating the expression of transforming genes. By contrast, chromosomes of group 9, which are present in revertant cells but not normal or transformed cells, probably carry factors which suppress the expression of malignancy. Because the appearance of group 9 chromosomes is associated with a decrease in the number of chromosomes in groups 6 and 7, Sachs and his colleagues suggest that the group 9 chromosomes of revertants are formed from groups 6 and 7 chromosomes, the latter bearing suppressor factors in normal and malignant cells.

Needless to say, experiments of the sort reported by Sachs and his colleagues depend absolutely on the precision with which individual chromosomes can be distinguished. And no doubt they would be among the first to admit that the currently available techniques for staining chromosomes to reveal their individuality leave much to be desired; a glance at the figures which illustrate their report and those of Rowley and Bodmer and Yunis and his colleagues (see pages 503 and 532) in this issue of *Nature* is enough to convince anybody of the inherent tedium of such experiments. New techniques are, however, coming along which should increase the certainty with which any particular chromosome can be picked out.

Rowley and Bodmer, for example, report the correlated use of three different staining techniques to identify the characteristic distribution of regions of heterochromatic, satellite DNA along the length of individual mouse chromosomes. Staining with aceto-orcein reveals the centromere and other characteristic constrictions along the arms of chromosomes. Fluorescence microscopy of mouse chromosomes stained with quinacrine mustard, a technique Caspersson and his colleagues have developed, reveals the centromere and these same regions of the chromosome as bands of reduced fluorescence. This technique depends on the binding of quinacrine mustard, which can be made to fluoresce, to guanine residues and so it reveals reproducibly regions of the chromosome rich or poor in this base.

Finally, Rowley and Bodmer have denatured and re-natured chromosomal DNA *in situ* using the same preparations that they previously stained with quinacrine mustard. They then stained the cells with Giemsa; the centromere and the same heterochromatic regions which had been revealed by the two other techniques were again revealed, this time more intensely stained than the rest of the chromosome.

These three methods reveal the pattern of distribution of heterochromatic, satellite DNA, in other words the signature, of individual chromosomes. And as Rowley and Bodmer comment, the fluorescent banding technique, in particular, should prove to be extremely valuable in following the chromosomal evolution of cells in culture and in "identifying those mouse chromosomes which may carry genes for repressing malignancy". That is something which Sachs and his colleagues and others will doubtless be quick to follow up.

OCEAN RIDGES

Anomalous Uranium

from our Geomagnetism Correspondent

THE recent discovery by Boström and Fisher (*Nature*, **224**, 64; 1971) of unusually high uranium concentrations in East Pacific Rise sediments has inevitably raised the question of the origin of the "excess" uranium. Could it, for example, have been derived from volcanic processes? The first thing to be said about this hypothesis is that there is not a shred of direct evidence for it. On the other hand it is known that volcanic emanations have enriched ridge crest sediments in elements such as iron and manganese; and this could be taken as circumstantial evidence for volcanic uranium enrichment. There are, however, other possible sources for the excess uranium. Ku, for example (in *Hot Brines and Recent Heavy Metal Deposits in the Red Sea*, Springer, New York, 1969), recently suggested that the high concentrations of uranium in iron-rich deposits from the Red Sea geothermal area might be attributable to the deposition of uranium from seawater by coprecipitation with ferric iron.

Veeh and Boström (*Earth Planet. Sci. Lett.*, **10**, 372; 1971) now suggest that Ku's mechanism may well be applicable to the East Pacific Rise sediments; and from a new series of analyses of sediments and iron deposits show that this is highly likely in most—but not all—cases. The $^{234}\text{U}/^{238}\text{U}$ ratio in seawater is constant at 1.15 ± 0.02 , so that measurement of this ratio in newly formed sediments should presumably reflect this value in some way. In fact, in all but one of Veeh and Boström's samples, $^{234}\text{U}/^{238}\text{U}$ ratios lie between 1.00 and 1.14—in other words, they are less than but approaching the constant oceanic value. These compare with values of unity or less for pelagic clays but are in line with values from the Red Sea geothermal deposits. A seawater origin thus seems reasonable.

Unfortunately, seawater cannot be the whole story, for one iron deposit dredged from the flank of a seamount on the crest of the East Pacific Rise has a $^{234}\text{U}/^{238}\text{U}$ ratio of 1.21. Strangely, Bonatti and Joensuu (*Science*, **154**, 643; 1966) ascribed this iron deposit to submarine volcanism, probably produced by an interaction between hydrothermal solutions and seawater. Yet, clearly, its uranium cannot be derived completely from the seawater. In this one case therefore volcanic processes become a real possibility, although, as Veeh and Boström point out, one of at least two different volcanic processes could be applicable.

The first appeals to fractionation of uranium isotopes during volcanic activity. Fractionation is quite com-

mon in weathering processes and seems to be the principal cause of the 15 per cent excess of ^{234}U over ^{238}U in seawater. It turns out, however, that it would not be possible to derive all of the excess ^{234}U by such a mechanism; and to overcome this objection Ku suggested (*J. Geophys. Res.*, **70**, 3457; 1965) that additional ^{234}U might be derived by the preferential leaching of ^{234}U from deep sea sediments and its subsequent diffusion into the bottom waters. Whether the sort of fractionation processes which take place during weathering also take place during magmatic activity is unknown, however, though they remain a possibility.

In fact, an appeal to magmatism as such is unnecessary. Thus Veeh and

Boström suggest the preferential leaching of ^{234}U from wall rocks by rising hydrothermal fluids associated with submarine volcanism. The uranium is then coprecipitated with ferric iron upon contact with seawater. In general the uranium derived from seawater in the normal way will mask that produced by the hydrothermal process because of rapid mixing of the fluids with the bottom waters. But the question that Veeh and Boström ask is: could the iron be precipitated so quickly that such mixing would not have time to take place? They suppose it could—and thus that the precipitating iron could "grab" the hydrothermal uranium before it becomes dispersed in the seawater.

Angles on Pulsars

Two contradictory views of the same problem—the emission mechanism of the pulsar in the Crab Nebula—are published in next Monday's *Nature Physical Science*. Both articles include as evidence the characteristic polarization of the three subpulses that make up the pattern of emission from the pulsar at optical and radio wavelengths. And both are founded on the view that the Crab Nebula pulsar is a rapidly rotating star having a dipole magnetic field oriented at an angle to the rotation axis. R. N. Manchester of the National Radio Astronomy Observatory, however, argues that the magnetic and rotation axes are at right angles, whereas in the following article F. G. Smith (Jodrell Bank) concludes that the angle between them is likely to be about 45° .

The chief difference between their approaches is that Smith concentrates on a relativistic approach already set out in detail in *Monthly Notices of the Royal Astronomical Society* (**149**, 1; 1970). In brief, Smith has been canvassing support for the view that the source of the pulsar emission is radiating isotropically in its own frame of reference, but which appears to be emitting a beam when viewed from the Earth. The relativistic velocities needed for this mechanism are acquired by sources rotating with the star, but some distance from it; the Crab pulsar completes one revolution every 33 ms, so the possibility of material spinning with the star at a tangential velocity approaching c has to be taken into account.

Next Monday Smith takes the story a step further when he shows that relativistic effects should also be allowed for in evaluating the expected position angle of linearly polarized components of the emission. His interpretation of the quite characteristic swing of the polarization through the pulses which is observed leads to the view that the

radiating source is located away from the polar magnetic field lines, that the line of sight to the pulsar is at an angle of 20° – 30° to the rotation equator, and that the axis of the magnetic dipole is inclined at something like 45° to the rotation axis.

But in the preceding article Manchester bases his interpretation of the polarization measurements on the view that the optical and radio emission both come from nearer the surface of the star, and in the vicinity of the magnetic poles rather than away from them. He argues that both the optical and the radio signals are emitted essentially tangentially to the field lines, so that at the peak of the pulses the line of sight must be almost directly down onto the magnetic pole. The way in which the position angle alters across the pulses in different ways at optical and radio wavelengths is accounted for by the basic synchrotron emission arising in different ways in the two bands.

The pattern of the variation in position angle of the optical pulses also suggests to Manchester that the orientation of the field lines in the region where the optical radiation is being emitted is slightly different from the orientation associated with the radio emission. Possibly the optical emission is occurring further out from the surface of the star, but nevertheless still along the same polar field lines as the radio emission, in contrast to the argument in Smith's article that the source is away from the polar field lines.

Manchester goes on to point out that if the radio and optical pulses come from different regions above the magnetic poles, and if the emission is tangential to the field lines, then in the case of the Crab pulsar radio and optical pulses will only be seen together if the rotation axis is at right angles to the magnetic axis.

SLEEP

Innate and Unmodifiable

from our Experimental Psychology
Correspondent

THERE used to be quite a good way of starting an article on sleep by saying that although sleep occupied about a third of our lives there was almost nothing known about it. That situation has changed and more and more work is being done on the subject. Recent experiments by one of the best known sleep researchers, W. B. Webb of the University of Florida, and his associate J. Friedman (*Physiol. Behav.*, **6**, 459; 1971) add further support to the view that sleep is an innate pattern not capable of any permanent modification by experience within the life of the individual.

Webb and Friedman attempted to modify sleep patterns of rats in three ways. First they kept rats in an ordinary fixed cage, restricting running activity, from weaning until 90 days of age, and compared these animals with rats that had been kept for a similar period with access to a running wheel. Second, they kept rats awake for either 6 or 12 h a day for 30 days on a slowly moving wheel which dipped into water. Third, they woke rats with a mild electric shock every time they fell asleep for 6 h a day for 10 days. No differences of any kind in the total amount of sleep or the percentage of paradoxical, rapid eye movement sleep (the phase which in man is accompanied by dreaming) were found between experimental and control animals when they were tested at various times after any of these procedures. Evidently, in spite of energetic measures of behavioural control, the innate sleeping pattern persisted; a finding not without significance for many shift workers, airline pilots, seamen keeping 4 h watches and the like.

C. P. Richter has also come to a closely related conclusion (*J. Comp. Physiol. Psychol.*, **75**, 1; 1971). He found that rats blinded soon after birth before there was any functional vision, and congenitally blind rats, develop distinctive rhythms of sleeping and waking just like sighted animals. Being sightless, though, these rats are not synchronized to the lighting cycle, but exhibit their innate endogenous rhythm with periods near 24 h.

These demonstrations of the strong innate patterning of sleep are consonant with the notion that the phenomenon has evolved because of its survival value. Animals that depend on vision are both vulnerable and powerless in the dark and sleep, in producing inactivity and quiet, may simply be the biological response to this selective pressure. If so, it seems that man is stuck with a mechanism which is at the

same time peremptory, largely immovable, and serves no particular purpose in an electrically illuminated civilization.

HERPES VIRUSES

Elusive Carcinogens

from our Cell Biology Correspondent

IN spite of the vast accumulation of circumstantial evidence of one sort or another, all of which suggests that certain herpes viruses may induce malignant tumours in man and some other species, an unequivocal proof of this claim seems to be almost as far away as ever. To be sure, nobody nowadays seriously disputes that Marek's disease of chickens is caused by a herpes virus but pathologists are not agreed as to whether or not this is really a malignant disease. Attempts to protect populations at high risk to Burkitt's lymphoma with EB virus vaccines have yet to begin, and, to judge from recent reports, the herpes virus which is present in the cells of the Lucké kidney tumour of the frog *Rana pipiens* is proving to be remarkably uncooperative when attempts are made to propagate it *in vitro*. The Lucké adenocarcinoma, however, still offers what seems to be the best opportunity to test the oncogenic potential of a herpes virus.

There are two well established and salient observations. First, the cells of this adenocarcinoma of frogs contain a herpes-type virus (L-HTV) if the

animals are collected from the wild in winter or are maintained in the laboratory at 4–8° C. By contrast, no virus can be seen in the tumour cells of frogs collected in the summer months or maintained in the laboratory at 20–25° C. But as Mizell and her colleagues have shown when pieces of such summer phase tumours are transplanted into the eye chambers of *Rana pipiens*, *Bufo americanus* or *Rana clamitans* (the latter two species do not naturally contract the Lucké adenocarcinoma) held at 4–8° C, virus appears after about two months. Mizell's group have also now achieved the induction of the herpes virus *in vitro* by maintaining explants of summer phase tumour on agar slants at 7.5° C for up to 14 weeks (Breidenbach *et al.*, *J. Virol.*, **7**, 679; 1971). That Lucké adenocarcinoma cells are infected with a persistent herpes virus does not, however, prove anything. For, as anybody who suffers from recurrent cold sores knows, herpetic infections have the habit of persisting often for the lifetime of the individual, periodically becoming manifest as a result of all manner of unusual stimuli.

The acid test, of course, is to purify the Lucké carcinoma virus, inject it into embryonic or adult frogs and see if they develop adenocarcinomas. But unfortunately, Lucké carcinoma cells usually contain other viruses in addition to the herpes virus, and uncontaminated preparations of the Lucké virus, which will transmit the tumour, have so far not been obtained. Furthermore,

Condensation of Hydrogen onto Interstellar Grains

THE nature of interstellar grains remains a mystery in many ways, because so little is known about the properties of small grains and their interactions with other grains and molecules under the conditions of interstellar space. This has left plenty of room for theoretical manoeuvring and a variety of theories of various degrees of plausibility have been produced. One of the most widespread ideas—or rather one which has at least achieved the most prolific publication rate—is that hydrogen might freeze onto interstellar grains, with important astronomical consequences (see, for example, the summary in *Nature Physical Science*, **231**, 115; 1971). The chief proponents of this idea have been V. C. Reddish, of the Royal Observatory, Edinburgh, and N. C. Wickramasinghe, of the Institute of Theoretical Astronomy, Cambridge. In collaboration with T. C. Lee and L. Gowland, Reddish now reports, in the forthcoming issue of *Nature Physical Science*, experimental results which seem to confirm his theoretical ideas.

Experimental studies of the vapour pressure of solid hydrogen and both hydrogen–nitrogen–oxygen and hydrogen–helium mixtures carried out by Lee *et al.* show that impurities present at the levels expected in interstellar space do not significantly affect the condensation of hydrogen onto grains. There is, unexpectedly, a large thermal impedance between the hydrogen layer; the grain on which it has condensed is found below 3 K, and this could result in a temperature difference of as much as a few tenths of a degree between the grain and its hydrogen mantle, which will undoubtedly affect the radiation, absorption and emission properties which are so important to the study of interstellar clouds and the interpretation of observations. But the most important deduction reached by this team remains that if grains cool to the temperature of the cosmic background radiation, then hydrogen will freeze onto them in clouds with densities of hydrogen molecules greater than 10^{13} m^{-3} .

attempts to propagate the Lucké herpes virus in such preparations on cells in tissue culture have failed, resulting only in the new isolation of the various contaminating viruses.

One of these, frog virus 4, or FV4, which was originally isolated by Rafferty from the pooled urines of tumour bearing frogs, is a herpes virus which can be propagated in tissue culture cells, but it does not induce Lucké carcinoma. And according to Gravell (*Virology*, **43**, 730; 1971), FV4 is quite distinct from the Lucké virus. The DNA of the former, for example, has a melting temperature of 91.5° C whereas the Lucké virus DNA melts at 87.6° C. Further, hybridization experiments indicate little homology between the two DNAs and neutralization tests indicate that the two viruses are not serologically related. It seems therefore that the viruses which can be isolated from frogs bearing Lucké adenocarcinomas and cultivated *in vitro* are either not involved in the induction of the carcinoma, or play only a helper part. The problem of obtaining pure Lucké virus remains as intractable as ever.

PROTEINS

Umbrella Enzyme

from our Molecular Biology Correspondent

It is a characteristic feature of work on cooperativity in subunit enzymes that a small input of experimental data yields wholesale returns in theoretical vapourings. It is the more refreshing therefore to come upon a paper in which the results are so direct and apparently so conclusive that they can be allowed to speak for themselves.

It is now five years since Kirschner and his colleagues published their first study on the kinetics of the interaction of yeast glyceraldehyde-3-phosphate dehydrogenase with its cofactor, NAD. The issue to which they chiefly addressed themselves was whether the cooperative binding profile arose from a concerted change between two conformational arrangements (as envisaged in the original formulation of allosterism) or by a stepwise mechanism, in which a ligand-induced change in one subunit induces a switch from one state to another in its neighbours (the sequential model of Koshland, which traces its ancestry back some thirty-five years, to Adair), or something in between, which is a possibility that a completely general scheme must embrace.

Kirschner has elsewhere presented the umbrella as a homely model for the first type of mechanism: the structure can turn inside out in a concerted manner, all subunits remaining equivalent,

whereas the sequential change, one rib at a time, is impossible, hybrid states being sterically forbidden. What the early work indicated was that the binding of cofactor by glyceraldehyde-3-phosphate dehydrogenase followed the predictions of the concerted model. There were, however, features of the kinetic data that could not readily be fitted into the scheme, and a more complete study with the aid of better apparatus, developed in the interim, and of a more secure grip on the chemistry of the enzyme, has now at last appeared.

Kirschner *et al.* (*J. Mol. Biol.*, **58**, 29; 1971) begin with a re-examination of the equilibrium binding curves. The enzyme has several curious features, one of which is that cooperativity is observed only under narrowly defined conditions

(including a temperature of 40°). In the first place they find that under these circumstances the absorption band of NAD, the change in which on binding is used as a measure of fractional saturation of the protein sites, has a sizable temperature dependence. This effect must therefore be taken into account in the analysis of the temperature jump kinetics. Second, the possibility that the shape of the binding curve is affected by failures of the absorption change to follow the degree of saturation is ruled out by comparison with equilibrium dialysis measurements. The presence of a minor component in the crystalline enzyme was also established, and it was separated by column chromatography.

The four processes previously recognized in the temperature-jump relaxa-

Recombination between Mouse and Chick DNAs

If genetic engineering is to be anything more than the fantasy of the writers of science fiction, ways of promoting the integration of foreign DNA into the genomes of recipient cells will have to be devised. To be successful, the genetic engineer will have to be able to control recombinational events between the DNA he wishes to introduce into a cell and that cell's genome. That is obviously a very tall order, but the remarkable experiments reported by Hill and Hillova in next Wednesday's *Nature New Biology* suggest that it may be possible to introduce and recombine foreign DNA in mammalian cells.

Like many another group, Hill and Hillova have been trying to prove that a foreign DNA—they used mouse DNA—can be incorporated into the DNA of a recipient cell, in their case chick cells in culture. Clearly there are several potential methods for detecting the permanent association of a foreign DNA with a recipient cell. One obvious approach is to try to introduce a piece of foreign DNA which specifies an enzyme; its presence can then be detected by showing that the recipient cell acquires this new enzyme, characteristic of the donor species. But rather than do that, Hill and Hillova have taken the bull directly by the horns, exploiting radioisotope and density labels and centrifugation in neutral and alkaline sucrose gradients in an attempt to show that small segments of mouse DNA are incorporated into molecules of chick DNA.

They simultaneously exposed their cultures of chick cells to mouse DNA labelled with tritium and the base analogue bromodeoxyuridine (BUDR) labelled with carbon-14. The cells were exposed to this mixture for some 20 h during which time many replicated their DNA. Any newly synthesized strands

of chick DNA made in this time should contain BUDR labelled with carbon-14, because this compound is incorporated in place of thymidine residues. Furthermore, newly made chick DNA should contain some bases labelled with tritium because some of the mouse DNA is bound to be degraded so that its bases will enter the pools of precursors from which new chick DNA is made.

Because BUDR is denser than thymidine, any DNA containing appreciable amounts of this analogue can be separated from thymidine containing DNA. This fact enabled Hill and Hillova to separate first the newly replicated double helices, one strand of which contained BUDR and the other, the template, contained only thymidine, from unreplicated DNA both strands of which contain only thymidine. They were then able to separate the two strands of newly replicated DNA, because only one contains the density label BUDR.

In short, what they found was that some of the newly replicated strands of chick DNA were less dense and contained more tritium labelled nucleotides than they anticipated. They also found that a small fraction of the mouse DNA introduced into the chick cells had not only survived but had become some 16 per cent denser.

The conclusion is obvious enough: it seems that a small percentage of the newly replicated strands of chicken DNA contain intact segments of mouse DNA. If this really is the case, and Hill and Hillova had to push the methods they used to the very limits of their resolution, some recombination must have occurred between the chicken and mouse DNAs leading to the integration of intact short segments of mouse DNA. The long term implications of this phenomenon are clear.

tion experiments on the dehydrogenase-NAD system can now be assigned. There is an immeasurably fast initial change, which corresponds to the effect of the temperature rise on the NAD spectrum. Of the two second-order processes one (the slower) can now with certainty be attributed to the minor component and is absent in the purified protein. This leaves one other relaxation, the concentration-dependence of which shows it to be first order.

The NAD binding process therefore involves only one rapid second-order and one slow first-order reaction. The sequential binding scheme, with a set of consecutively different affinities, has no room in it for a first-order reaction. The concerted model on the other hand demands an intramolecular isomerization between states of high and low binding affinity; moreover there is also as expected a sharp decrease in this first-order rate constant as the NAD concentration increases. Other criteria for an all-or-none affinity change are likewise fulfilled, and it is also found that all four subunits must participate in the cooperative process, so that models based on dimeric cooperative units can be excluded.

Although these results make possible by far the least equivocal assignment so far of a mechanism for a cooperatively interacting system, one would not of course try to generalize from this one case, and indeed Kirschner *et al.* point out that the conditions in the cell are very likely such that the cooperativity is not called into play. If this is so, the teleological argument would run, the mechanism in an enzyme designed for, rather than adventitiously endowed with, cooperativity might well be quite different.

In a companion paper (*ibid.*, 51) Kirschner has used the stopped-flow method to study the properties of the NAD-complex of the enzyme in both its affinity (allosteric) states. This is made possible by the slow rate of conversion of the apoenzyme from the unreactive state, in which under the conditions of the experiment it predominantly finds itself, to the reactive state on addition of NAD. In effect a metastable complex of NAD with the low-affinity state is formed, which endures for more than 3 s. The spectroscopic change accompanying the binding of NAD to the low-affinity state was determined, and the activity of this form, with the NAD attached, towards the substrate glyceraldehyde-3-phosphate could also be studied. This proved to be negligible. Kirschner shows that with the aid of this interesting and important fact, earlier kinetic data on the enzyme, previously apparently hopelessly complicated, can be quantitatively explained.

SELENOLOGY

Why Lunar Erosion?

from our Geomagnetism Correspondent

THE presence of soils and fines on the Moon's surface indicates that the lunar rocks have undergone extensive erosion. But what is the predominant erosion mechanism? The lack of water on the lunar surface shows that one of the most important causes of terrestrial erosion cannot apply to the Moon; but the existence of so many lunar craters, on the other hand, has frequently led to the assumption that the principal source of erosion is meteorite impact, or at least the secondary ejecta thrown up by the impact of meteorites. From an examination of the morphology of microcraters in lunar rocks, however, Hörz *et al.* (*Earth Planet. Sci. Lett.*, **10**, 381; 1971) now conclude that although the occurrence of erosion by meteorite impact is not in doubt it is not a very significant process—the bulk of lunar erosion seems to take place not as a result of the impact of conventional meteorites but of primary cosmic ray particles.

To arrive at this conclusion Hörz and his colleagues carried out detailed stereoscopic microscope investigations of about 4,000 lunar microcraters larger

than 0.4 mm. The samples examined were two fine-grained and three coarse-grained crystalline rocks and one breccia from the collection of Apollo 12 samples.

Precise details of the morphologies of the microcraters are to some extent dependent on the particular type of rock; but most craters examined comprised a central pit (a glass-lined cavity in the centre of the overall crater), a surrounding halo (a concentric zone of shock-fractured minerals) with a high albedo and an average diameter 2.2 times greater than that of the pit, and a spall area (a concentric area around the pit which was removed by shock wave interaction with the free surface) which includes the halo and whose average diameter is 4.5 times greater than that of the pit. Completely fresh pits have diameter-depth ratios ranging from 2 to 5. Variations in relative diameters and depths are undoubtedly attributable to differences in impact velocities, the intensity of fracturing of the rock surface from previous impacts, the relative abundances of target minerals and errors of measurement. The point is, though, that almost all microcraters have the same morphological form.

The geometries of the microcraters are apparently similar to those of cra-

Induced Self-fertility

IN these days of technological precision it is comforting to find that results may still be obtained by accident. A case in point is the work reported by Brasier in next Wednesday's *Nature New Biology*. He has found that sex organs can be induced in heterothallic fungi when normally they will arise only when isolates of different compatibility groups are paired. Thus, sex organs were induced in an isolate of *Phytophthora palmivora* when contaminated with an isolate of another fungus, *Trichoderma viride*.

Screening of many different species of *Phytophthora* followed and the stimulus provided by *Trichoderma* was found to be effective in nearly all cases, although of the two sexually compatible groups, A¹ and A², only A² demonstrated this induction of the sex organs and the effect seems to be confined to isolates of this group.

The chemical nature of this sexual stimulus cannot yet be defined but the restriction of the stimulus to A² suggests that it may have affinities with substances produced by the A¹ compatibility type. Although Brasier could not induce sexuality with liquid culture filtrates or autoclaved agar cultures, he found that production of sex organs was stimulated by inverting a Petri dish of *Trichoderma* over a

Petri dish of *Phytophthora* so that there was no physical contact between the two cultures. It was observed that a far higher proportion of *Phytophthora* sex organs contained mature oospores when there was no physical contact, probably because the more rapidly growing *Trichoderma viride* killed the *Phytophthora* over which it grew. It seems likely, however, that the chemical responsible for the stimulus must be volatile.

Brasier points out that observations of a similar nature have been made in the past few years for bacteria and metabolic diffusates of soil microorganisms have also been shown to cause the sexual stimulation of some species of *Phytophthora*. His own attempts with a number of other soil fungi have so far proved unsuccessful.

Brasier's finding has important genetic and ecological implications quite apart from its obvious use of making the identification of heterothallic *Phytophthora* easier. The possibility of inducing selfing in A² isolates would render genetic analysis much more simple and if the phenomenon occurs in nature, where *Trichoderma* and *Phytophthora* are potentially antagonistic, the survival of some *Phytophthora* species as oospores may be considerably enhanced.

ters produced on glasses and crystalline materials in the laboratory with projectiles the velocities of which exceed 10 km s^{-1} . Because of this, and because of the presence of a glass-lined pit in most lunar microcraters, Hörz *et al.* conclude that the velocity of 10 km s^{-1} must also be regarded as the minimum velocity for the production of these microcraters. On the face of it then such microcraters could be produced by the ejecta of a large scale impact event, for Gault *et al.* (in *Shock Metamorphism of Natural Materials*, Mono Book Corporation, 1968) recently showed that the velocities of such ejecta may well reach 20 km s^{-1} . The velocities of fine-grained particles within any given impact "jet" are unlikely, however, to exceed 10 km s^{-1} . The only other particles impinging on the Moon with greater velocities are primary cosmic rays; and so Hörz and his colleagues are led to the conclusion that at least 95 per cent of the lunar microcraters are produced by cosmic ray impact.

But velocities are not the only consideration. Three of the crystalline rocks examined by Hörz *et al.* have sharp boundaries between heavily cratered and completely uncratered surfaces. In theory, of course, this phenomenon is quite easy to explain in terms of the effect of secondary ejecta—uncratered areas could be taken to be newly broken surfaces not yet exposed to the bombardment of secondary projectiles. If this were so, however, boundary lines would be associated with the sharp edges and corners characteristic of breaks.

But in the lunar rocks examined there is no such correlation. On the contrary, in all three samples the boundaries between heavily cratered and uncratered areas run across relatively flat surfaces and bear no relation at all to rock geometry. Moreover, the boundaries are planes rather than lines in that they can be traced around the whole circumference of the rocks. As a result of these features, Hörz and his colleagues suggest that the uncratered surfaces were simply buried in the lunar regolith and thus remained unexposed to cosmic rays.

Finally, as Hörz *et al.* point out, the presence of a spall zone around the crater pits shows that the predominant erosion process on the scale observed is the "catastrophic rupture" of rock surfaces by discrete microfracturing events. Overlapping spall zones are frequent, the complete or partial destruction of craters by individual events is commonly observed and discrete pieces of pit walls are often seen to be broken off. All these features indicate not the gradual removal of material but catastrophic removal by high velocity, high energy impacts.

INSECTS

Cricket Songs

from a Correspondent

ACOUSTIC biology was, as expected, the chief (but not sole) concern of a semi-ACOUSTIC biology was, as expected, the insect body and between insects held in the Animal Acoustics Unit of the Sir John Cass School of Science and Technology (City of London Polytechnic) on June 9 and 10.

After a short film of bush-cricket stridulation (Mr A. Currie, Sir John Cass), some physical aspects were discussed by Dr H. C. Bennet-Clark (University of Edinburgh) and Mr H. Nocke (University of Cologne). Comparing *Drosophila* (an acoustic doublet in a free field) with the mole cricket in its burrow (a piston-driven exponential horn) highlights their differing communication problems—*Drosophila* loud,

but short range and private, *Gryllo-talpa* long range and broadcast. In *Gryllus*, Nocke's elegant series of experiments shows unequivocally that the "harp" of the tegmen (not the "mirror") is the radiator—a linear-type resonator uniformly tuned; and that it can also resonate to the emission of another cricket, as far away as 150 cm.

The discussion of hearing by Professor F. Huber (University of Cologne) and Mr D. B. Lewis (Sir John Cass) started with song recognition and its neuronal basis, and the control on responsiveness. Regen's classic work was always misinterpreted—he never claimed to have found clear frequency discrimination. Nevertheless, physiological evidence of some possible degree continues to accumulate, even to the level of processing in the brain. In intensity/time studies, an important new finding is that at the cellular level,

Greater Complexity in the Mantle

ONCE upon a time the upper mantle seemed to be a remarkably homogeneous region within the Earth. But as seismic techniques have gradually become more refined the upper mantle has gradually come to appear less and less homogeneous. So many discontinuities and partial discontinuities have now been observed, often quite close together, in the upper 1,000 km of the mantle that it is no longer possible to be sure that the boundary one seismologist observes below one part of the Earth's crust is the same as that observed by another worker at a roughly similar depth below a different part of the crust. Nor is it clear that any given discontinuity actually exists around the whole Earth whether at a constant or varying depth. In short, the picture gradually becomes more confused; and in next Monday's *Nature Physical Science* observations reported by Simpson *et al.* apparently add to the confusion. This is not, of course, an adverse criticism of these authors' work but merely a reflexion on the complexities of nature.

Simpson and his colleagues have taken advantage of the greater resolution available from combining seismic array data (P wave travel-time gradients) with conventional travel-time data to discover a previously unknown segment of travel-time curve. The array in question was WRA (Australia); and 330 earthquakes within the Indonesian-Philippines region (epicentral angles 12° to 30°) were analysed. The uncorrected P wave travel-time gradients clearly indicate the presence of upper mantle discontinuities at 360 km and 650 km—discontinuities which have been observed previously by several

other seismologists. But between epicentral angles of 19.4° and 20.6° Simpson *et al.* have detected, for the first time, several low amplitude arrivals a few seconds earlier than the times predicted from a simple model involving only the two discontinuities at 360 km and 650 km. In the sense that these newly discovered arrivals arise from epicentres within a very limited region (the Molucca Passage), they may just be a reflexion of crustal structure, even though Simpson and his colleagues have attempted to correct for such structure. Simpson *et al.*, however, adduce other evidence which suggests that this problem does not arise here and, as a result, conclude that the 360 km discontinuity should be replaced by a doublet at 280 km and 440 km.

How then does the new Simpson model (discontinuities at 280 km, 440 km and 650 km) compare with others? The closest perhaps is that deduced recently by Whitcomb and Anderson (*J. Geophys. Res.*, **75**, 5713; 1970) who, using a completely different method, observed discontinuities at 280 km, 520 km and 630 km. This is a pretty good correspondence for the upper and lower boundaries but not for the middle one. Although Whitcomb and Anderson observed no obvious boundary at 400–450 km, however, there is ample evidence for such a discontinuity which has even been interpreted in terms of a phase transformation from olivine to a spinel structure. The point is that the existence of Simpson's 440 km discontinuity is well supported by other workers. On the other hand, the 520 km boundary does not appear in the Simpson model. The discrepancies between the two models thus remain.

transients can be coded by an enhanced tendency to synchronize single-element discharges, rather than by special phasic receptors. The significance of the ascending and descending messages in the prothoracic T-fibres perhaps needs solution. But reception begins even before the sense cells: the great prothoracic spiracle of the bush cricket, long overlooked, which passes straight down to the back of the eardrums approximates almost exactly to an exponential horn, and leaves the function of the tiny accepted earflits entirely problematical.

Two striking features of brain anatomy, according to Dr P. E. Howse (University of Southampton), are the relative isolation of the central complex which suggests a buffered steady-state function, and the way the addition of visual memory to sociogenic processes seems to have affected the size relationships of mushroom bodies and calyces. The antennal cleaning reflex (Mr M. O'Shea, University of Southampton) provides a useful simple model of motor-output pattern control by the central nervous system; in the more complex stridulation behaviour (Huber), a central thoracic oscillator (or oscillators) now seems likely, with a limited repertoire of output rhythms programmed by various inputs, including both ascending and descending elements, the switch depending at least partly on differential thresholds for different behaviours, perhaps not at all on locus in the brain. The role of endocrines in the whole behaviour sequence (another area of equivocal results in the past) bids fair to be cleared up by the painstaking work in the Hebrew University of Jerusalem, by Dr M. P. Pener and Miss E. Wajc, in bringing rigour to the measurement of behavioural results of extirpation/implantation of pars intercerebralis and corpus allatum. The information systems controlling egg diapause in bush crickets (Dr J. C. Hartley, University of Nottingham) evoked considerable questioning and there was appreciation of the light that this work has thrown on laboratory culture of these necessary animals.

Effects of sound reception on production in Orthoptera were reviewed by past and present members of the Animal Acoustics Unit. Dr M. D. R. Jones (Brunel University) has now established that the inhibition mechanism controlling alternate singing of conspecific males has a graded resetting action on the timing of chirps, depending on the point of entry of the "intruding" signal; in *Jamaicana*, it may also affect the syllable oscillator—a new result. Other members outlined follow-ups of Broughton's original discovery of song mutilation between species.

METEOROLOGY

Sea Ice Terminology



A light nilas—a thin elastic crust of sea ice more than 5 cm thick—one of many terms defined and illustrated in a new edition of *WMO Sea-ice Nomenclature* (World Meteorological Office: Geneva).

Mitochondrial RNA Polymerase Purified

ALTHOUGH mitochondria are as dependent on nuclear genes for some of their structural proteins as the rest of a cell is dependent on mitochondria for a supply of ATP, there is ample biochemical evidence to conclude that these organelles have evolved from symbiotic bacteria. Mitochondria, for example, have their own DNA genomes and a translation apparatus which closely resembles that of bacteria and is quite distinct from that of the cell cytoplasm. Mitochondria of the fungus *Neurospora crassa*, and of other organisms, also have an RNA polymerase which Küntzel and Schäfer have managed to isolate in a pure form. As they describe in *Nature New Biology* next week this enzyme resembles bacterial RNA polymerase in its sensitivity to inhibitors but in its structure it resembles more closely bacteriophage T7 RNA polymerase.

Küntzel and Schäfer have succeeded, where others have failed, in purifying this mitochondrial enzyme by not slavishly following the procedure used to isolate the bacterial enzyme—a procedure which inactivates the mitochondrial enzyme—but by adapting the methods used to isolate RNA polymerase from the nuclei of eukaryotic cells. And they have exploited the finding that the mitochondrial enzyme, once solubilized, aggregates in the absence of monovalent cations to form complexes with sedimentation coefficients in excess of 25S, to purify the enzyme.

The pure enzyme makes significant amounts of RNA in the absence of added DNA template so long as it is supplied with the four nucleoside triphosphates; this incorporation is, how-

ever, abolished by DNAase and so presumably some template DNA remains bound to the enzyme during purification. In spite of this endogenous template the addition of native mitochondrial DNA stimulates four-fold RNA synthesis; the enzyme uses with equal efficiency poly d(AT) but with less efficiency denatured mitochondrial DNA and it is inactive with native or denatured calf thymus DNA. Like bacterial RNA polymerase, the mitochondrial enzyme is inhibited by small doses of rifampicin but not by α amanitin; this pattern of response, of course, differentiates the bacterial and mitochondrial enzymes from their eukaryotic and bacteriophage T7 counterparts.

The physical properties of *Neurospora crassa* mitochondrial RNA polymerase are at first sight, however, something of a surprise. The enzyme is a single polypeptide chain weighing about 64,000 daltons whereas the bacterial enzyme consists of at least four polypeptide chains. Even bacteriophage T7 RNA polymerase, a single polypeptide chain, is, with a molecular weight of about 100,000 daltons, larger than the mitochondrial enzyme. As Küntzel and Schäfer point out on reflexion, however, it is perhaps not surprising that the mitochondrial enzyme is the smallest and simplest RNA polymerase known. Mitochondrial DNA cannot contain more than a few genes and a complicated set of positive and negative control proteins, to direct the selective transcription of particular genes, is not called for. A single repressor protein might suffice to regulate the transcription of mitochondrial DNA.

Proteins at Cold Spring Harbor

from a Special Correspondent

At the start of this year's Cold Spring Harbor meeting on the structure and function of proteins at the three dimensional level, M. Perutz (MRC Laboratory of Molecular Biology, Cambridge) asked whether protein crystallographic work was enjoying an expanding economy or whether it was entering a recession. In terms of the number and functional types of new protein structures completed and in progress, nobody can complain that output is not increasing, nor that the crystallographic technique is not being exploited.

Even during the meeting, Fourier maps were being calculated, and D. C. Wiley came panting from Harvard University with a 6 Å map of aspartate transcarbamylase. At high resolution this dodecamer of six regulatory and six catalytic subunits will pose (and answer?) exciting problems on the mechanism of control. As the tangled loops of yet more structures were revealed, the bewildering mass of information became more difficult to assimilate, and the still surprising details of active sites were looked at more and more narrowly. Anybody wanting to study protein architecture and folding in general terms will soon have more data than he can cope with. Almost the only contributor who asked general questions about protein architecture was F. M. Richards (Yale University), who—noting the historical swing from “polar groups want to be outside” to “hydrophobic groups want to be in the middle”—calculated the relative accessibility of polar and non-polar bits of a number of proteins. Unfashionable it may be, but his conclusion was that solvent can “see” polar and non-polar groups in roughly equal proportion, and suggested that proteins fold in the way they do more because of the need to pair H-bond donors and acceptors than the need to bury non-polar side chains. More refined calculations may move the proportions more towards the current view, but clearly the balance is a finer one than most people had thought.

Proteolytic Enzymes

Although the structures of the two major classes of the serine proteases—the mammalian and the bacterial—have been known for some time, it was gratifying to hear that the structure of subtilisin Novo (reported by W. G. J. Hol (University of Groningen)) is very close to that of subtilisin BPN' with an r.m.s. difference for the α -carbon atoms of only 1.1 Å, and that trypsin (reported by B. Stroud from the California Institute of Technology) is, *mutatis mutandis*, just like α -chymotrypsin. Crystallographic experiments aimed at solving mechanistic problems continue to be elusive for these enzymes, and the only such contribution came from R. Henderson (until recently at the MRC Laboratory of Molecular Biology, Cambridge), who reported that when the active-site His₅₇ of α -chymotrypsin is methylated on N₃, the catalytic activity is reduced by about 10⁴. The modified enzyme binds substrate just as well as the native protein, though both acylation and deacylation are slower. The interruption of the hydrogen-bonded quartet of active-site residues (Ser₁₉₅-His₅₇-Asp₁₀₂-Ser₂₁₄) thus only cuts the rate by three or four orders of magnitude. Surprisingly, the pH-dependence of the reaction is unaltered.

Other protease crystallographers produced evidence for the subsite specificity of these enzymes. The conformation of several peptide reagents covalently attached to His₅₇ in γ -chymotrypsin was reported by D. R. Davies (National Institutes of Health), who showed that with N-acetyl-Ala-Gly-Phe-, the Phe residue binds in the “tosyl hole”, and the peptide chain extends along the main chain of Ser₂₁₄-Trp₂₁₅-

Gly₂₁₆ of the protein, with H-bonds in an antiparallel β -pleated sheet arrangement. Only small changes are required to adapt this model to that expected for an acyl-enzyme derived from a peptide substrate. Using a similar range of chloro-ketones to label the equivalent His in subtilisin BPN', J. Kraut (University of San Diego) reported a very similar situation for this protease. Here the backbone of Ser₁₂₅-Leu₁₂₆-Gly₁₂₇ is hydrogen bonded to the extended peptide chain. Both sets of workers compared these data with kinetic results from peptidolytic reactions. It is remarkable that, in addition to the close similarity in the constellation of catalytic residues, these enzymes apparently have closely analogous ways of binding peptide substrates. It is not easy to see what were the selective pressures that caused the bacterial and mammalian enzymes to converge in binding modes as well as in catalytic terms. The position is made more difficult by the report from D. M. Shotton (University of Bristol) that elastase does not bind di-peptides and tri-peptides analogously to the above two enzymes. In this study the peptides were not covalently attached to His₅₇, and the interactions are less favourable than for γ -chymotrypsin and subtilisin BPN'. It may be that non-productive binding modes become more favourable for this system where the end of the inhibitor is not anchored.

R. Huber (Max-Planck Institute, Munich) reported the structure of the pancreatic trypsin inhibitor (Kunitz). The present structure is at 2.5 Å resolution. It seems that trypsin can cleave the Lys₁₅-Ala₁₆ bond of the inhibitor, but these groups are held in close proximity by the tertiary structure of the inhibitor molecule, and the equilibrium constant is well over in favour of the intact form. The inhibitor can be fitted nicely into the active site of trypsin when, once more, the specificity subsite interaction between inhibitor and the enzyme's main chain can be postulated.

A chemical contribution to the structure of the proteolytic enzymes came from F. H. Westheimer (Harvard University), who summarized work on the photolysis of diazoacetyl- and diazomalonyl-chymotrypsin and trypsin. The yields of photochemically generated carbene insertion products are very low, but the promise of the method was clear, and several coenzyme and other analogues were described. M. Dunn spoke on the spectra of chromophoric acyl-proteolytic enzymes (for S. Bernhard, University of Oregon), and concluded without much enthusiasm that the absorption spectra of these esters are more “ketone-like” than “ester-like”.

Overall, it seems that although several structural and specificity questions have been posed and some answered, the mechanistic details of this group of enzymes and the origins of the catalysis they perform remain much in the state they were a few years ago. This situation looks unlikely to change, unless there is more collaboration in the design of mechanistically meaningful experiments.

Glycolytic Enzymes

From X-ray structure work on glycolytic enzymes it began to look as if the glycolytic pathway ran between Bristol and Oxford. H. C. Watson (University of Bristol) showed diffraction patterns and crystal photographs of the five enzymes between glyceraldehyde phosphate dehydrogenase and pyruvate kinase, and of phosphoglucose isomerase, and reported on the progress of these structures. He stressed the importance of varying the enzyme source when scanning for crystallographically amenable crystals. P. Evans (University of Oxford)

showed a 6 Å Fourier map for horse phosphoglycerate kinase, and A. C. Bloomer (University of Oxford) reported progress beyond this resolution towards a high-resolution map for chicken triosephosphate isomerase. The change in unit cell dimensions when substrate or reaction intermediate analogue are diffused into the isomerase crystals was described, and it seems likely that even though the enzyme-substrate complex could require *de novo* solution, this would be done. Using evidence from isotope experiments on triosephosphate isomerase, J. R. Knowles (University of Oxford) led a conducted tour through the Himalayan ranges of its mechanistic pathway. The energetics of this enzyme were so defined that they led to a discussion of some experiments that the crystallographers might do. As with carbonic anhydrase (J. T. Edsall, Harvard University), it emerged that this enzyme may turn out to be one of what were called "Jencks's impossible enzymes" where the reaction rate is faster than the maximum predicted for normally dissociating acids.

Catalysis

The participants were denied (or spared?) a full round two of the orbital steering skirmish. In the absence of T. C. Bruice (University of California, Santa Barbara) the contribution by D. E. Koshland (University of California, Berkeley) on this subject was received rather quietly. Using evidence derived from lactonization of an extended series of norbornane derivations, he suggested that reactant site orientation could produce factors of 10^4 per reacting pair and allowed a 10° orientation of functionalities, which is more in line with known bond force constants. Elsewhere in organic chemistry, steric effects in the substituted 2,2,1-bicycloheptanes have provided fuel for considerable controversy during the past decade and it seems that these problems will now have to be faced also by biochemists. Among the doubters of orbital steering, W. P. Jencks (Brandeis University) rapidly summarized his views on the importance of proximity in enzyme catalysis and suggested that contributions of translational and overall rotational entropy have been underestimated. Even allowing for compensation from low-frequency motions in the products, some 35 enzyme units (equivalent to 10^8 in rate terms) remain as the entropy contribution to proximity. For most of his talk, however, Jencks focused on the range and meaning of the values of β from Brønsted-type correlations, and stressed the different extents of charge development in the transition states for different substrates and for different proteolytic enzymes. He argued cogently for the classical concept of general acid-base catalysis in such acyl-transfer reactions and showed that, in general, the enzyme-catalysed reactions suffer less charge development than non-enzymatic processes.

Two contributions took complementary viewpoints on where searching should start for the rate enhancements mediated by enzymes. G. E. Lienhard (Harvard University) is studying so-called transition-state analogues, the binding of which to enzyme active sites may be particularly strong. Thus a sugar lactone may fit more readily into the D subsite of lysozyme and have a near half-chair conformation which is suggestive of the proposed carboxonium ion intermediate. Similarly, an attempt to simulate the putative penta-coordinate phosphorus in the hydrolysis of 2',3'-cyclic UMP by ribonuclease A by using uridine + VO_2^{2+} was reported. R. J. P. Williams (University of Oxford) proposed that quite independently from lowering the energy of the transition state of the catalysed reaction, many metal-containing proteins lower the overall activating barrier by having ground states of higher energy attributable to asymmetric and strained coordination. This contribution catalysed considerable discussion and caused a certain amount of R to T state transitions among the participants.

Haemoglobin

Haemoglobin, described by one of those engaged in combat with this molecule (R. G. Shulman, Bell Telephone Labora-

tories, New Jersey) as "the common enemy", once again eluded her pursuers. In some confused fighting it seemed at times that more damage was done to the supposed allies than to the good ship haemoglobin. M. A. Raftery (California Institute of Technology) reported apparently conclusive fluorine nuclear magnetic resonance (NMR) studies showing that the responses of the CF_3COCH_2 -group, bound to the sulphhydryl group of the β -chains, indicated that in the reaction deoxy-haemoglobin to oxy-haemoglobin there is a conformational change which could be followed from chain to chain, that is to say, there is haem-haem interaction. The reaction of deoxy-haemoglobin to met-haemoglobin (Hill coefficient $n=1.2$ at pH 6.0) shows a pK_a around 7.5 not present in oxy-haemoglobin. This closely parallels the behaviour in unmodified haemoglobin. To some participants the result seemed to contradict the mechanism proposed as a result of the work of M. Perutz and the Cambridge group deduced from the crystal structure differences between deoxy- and aquo-met forms of several chemically modified and mutant haemoglobins. This work and that of Shulman on the NMR of mixed-state haemoglobins led to a re-affirmation of the no haem-haem interaction model. Many of the participants who have no direct interest in haemoglobin missed the last stages of the 5-hour session which had been preceded by 2½ hours of "informal" haemoglobin discussion on the beach. R. T. Ogata (Stanford University) used a spin-label in place of the 2,3-phosphoglycerate allosteric effector. The experimental results can be accounted for on the Monod model, but Ogata was not willing to press this interpretation. As far as the amateur is concerned the good ship haemoglobin has steamed far out of sight.

Metallo-proteins

The discussion of hydrolytic enzymes containing metals was enlivened by the chemical curiosities which seem to be present.

K. K. Kannan (University of Uppsala) reported on human carbonic anhydrase C. This protein, like many of those reported at this meeting, includes extensive regions of β -pleated sheets. The zinc at the active site of this enzyme has a roughly tetrahedral geometry with three histidines and one (?) water molecule as ligands. That in carboxypeptidase, described by F. A. Quiocho (Harvard University), is a distorted tetrahedron with two histidines bound through N(3) and one glutamate; and that in insulin (D. C. Hodgkin, University of Oxford) has a zinc that is probably six coordinate. These peculiarities could not be directly related to protein function. Water molecules at active sites are also seemingly in odd orientations, and S. H. Koenig (IBM Watson Laboratory, New York) went so far as to propose a liganded water in which one proton was very close to the metal. M. Cohn (University of Pennsylvania) demonstrated the use of NMR methods in the study of water relaxation rates in magnesium (replaced by manganese) enzymes. The problem of relating structure and mechanism was further compounded when B. L. Vallee (Harvard University) described spectrochemical probes for the active site tyrosine of crystalline carboxypeptidase A. It seems that the probe lies in the active site in the crystal, but out of it in solution (*pace* Lipscombe).

In the carp muscle albumin reported by R. H. Kretsinger (University of Virginia) the calcium ligands are three peptide carbonyls and a carboxylate; in staphylococcal nuclease they are three carboxylate groups. Two groups are at about the same stage in the analysis of concanavalin A (a manganese/calcium protein), but the state of the maps is such as to reveal only the subunit structure at present (K. D. Hardman (Argonne National Laboratory) and G. N. Reeke (Rockefeller University)). From low resolution crystallographic studies on tropomyosin with different amounts of bound troponin, D. L. D. Caspar (Children's Cancer Research Foundation, Boston) presented a trelliswork model of the organization of these materials in muscle.

Redox Systems

Studies by J. Kraut's group (University of California, San Diego) on high potential iron protein (HIPIP), and by L. H. Jensen (University of Washington, Seattle) on rubredoxin, have revealed the nature of some of the iron-sulphur sites. In rubredoxin a very distorted tetrahedron of cysteine residues bind the iron (III), with bond angles from 118° to 101° . Reduction does not cause major changes at the metal. There is one very short Fe-S distance (an Fe-S double bond?) and this sulphur, which is near the surface, could be implicated in oxidation-reduction reactions. The beautiful map presented by Jensen was at 1.5 Å resolution and his methods are the closest to small molecule work so far reported. Other proteins must be done to this resolution if bond length data are to be "accurate" (at 2.8 Å Jensen could not have stated with confidence that the tetrahedron was irregular). In HIPIP a rough tetrahedron of four iron atoms has four sulphur atoms on its faces and each iron is linked to one of four cysteines. As in cytochrome *c* and rubredoxin there are aromatic amino-acid side-chains running from the active site to the edges of the protein. Perhaps the electron transfer path runs through these residues. In these structures the temptation to look only at the "active site" is overwhelming, but many participants felt decided misgivings at their inability to know where else to look in the structure, and for what?

In the case of cytochrome *c*, R. E. Dickerson (California Institute of Technology) has now completed the structures of both the oxidized and the reduced forms at 2.8 Å resolution. The iron coordination remains unchanged (as was also elegantly shown by NMR studies of A. C. Redfield from the University of California, Berkeley), but there are large conformation changes around the iron and out to the periphery of the molecule. The cytochrome *b₅* peptide structure (at 2 Å resolution) done by F. S. Mathews (Washington University) shows the haem iron to be bound to two histidines. The haem is near the surface of the protein making contact with the solvent through a propionic acid side-chain. The peptide has a molecular weight of 11,000 to be compared with 16,000 for the protein. This protein is part of the multi-enzyme complex, microsomal NADH cytochrome *b₅* reductase. A different type of electron-transfer protein, flavodoxin, has been examined by M. L. Ludwig (University of Michigan) in all three oxidation states (the semiquinone form at 3.25 Å and the oxidized form at 4.0 Å), and it seems that the flavin changes its geometry with change in oxidation state.

A different group of redox enzymes which are usually oligomeric and more difficult to study are now yielding results. Heart muscle malate dehydrogenase (L. J. Banaszak, Washington University) and lactate dehydrogenase (M. G. Rossman, Purdue University) are well advanced at 3.0 Å and 2.5 Å resolution respectively. In these studies the structures of the bound NAD⁺ coenzyme have been seen and for LDH the abortive tertiary complex (NAD⁺ : pyruvate : LDH) has been taken to 3 Å resolution.

Immunoglobulins, Folding and Actinomycin

R. J. Poljak (Johns Hopkins University) has just obtained a 6 Å map of human myeloma F_{ab} fragments, and because the data are good to about 2.5 Å, a complete solution of this structure seems promising. The 6 Å map of a human myeloma IgG reported by V. R. Sarma (National Institutes of Health) is unfortunately unlikely to be taken much further, because the diffraction pattern falls off rapidly beyond 6 Å, and the crystals are very sensitive to radiation. Electron micrograph pictures of the crystal were presented to confirm the proposed (and almost expected) "Y" structure. A. B. Edmundson (Argonne National Laboratory) showed some promising diffraction patterns from crystals of both a serum myeloma IgG and the Bence Jones dimer from the same patient. Overall, it seems likely that the general architectural features, at least of the IgG molecule, will not be too long in coming.

A. Schechter (National Institutes of Health) presented some very clean 220 MHz NMR spectra of staphylococcal nuclease in the range of the acid-induced conformation transition, which, coupled with kinetic results on the fluorescence change of the single Trp in the molecule, began to point to a two (or at most three) state transition. In contrast, O. Jardetsky (Stanford Medical Center) has looked at the denaturation of this protein at high pH, and concludes that a sequential multi-state unfolding process occurs. Unfortunately, each group looked at residues which ionize (as well as change their environment) in the particular pH range, so the assignments of intrinsic *pK_a* (ionization) and apparent *pK_a* (folding) have to be sorted out.

One of the prettiest pieces of smaller scale crystallography was reported by H. Sobell (University of Rochester), who described the structure of the red 2 : 1 deoxyguanosine : actinomycin complex. The phenoxazone ring lies between the two nucleosides and Sobell postulated convincingly that actinomycin may intercalate into double-stranded DNA at p-G-p-C-sequences, giving rise to an attractive system of favourable interactions. As Perutz suggested, the details of this kind of study should fire the organic chemists to make antiviral agents that would intercalate in double-stranded RNA.

Viruses

Two highlights of the work on viruses were an analysis of the formation of the helix of RNA and coat protein in TMV, and a clear disagreement as to the conclusions which can safely be drawn from X-ray diffraction studies on spherical viruses. R. A. Crowther (MRC Laboratory of Molecular Biology, Cambridge) described the Fourier transform method for the three dimensional representation of electron micrograph pictures of icosahedral viruses at various tilt angles. A. Klug (MRC Laboratory of Molecular Biology, Cambridge) reported studies on TMV coat protein by both electron micrographs and X-ray diffraction and together with P. J. G. Butler (MRC Laboratory of Molecular Biology, Cambridge) proposed a convincing model for the formation of the RNA : protein helix from RNA and double disks of thirty-four coat protein units. The controlling influence of two anomalously titrating carboxyl groups on the polymerization was stressed. The RNA winds its way into disks, which dislocate stepwise from 17 protein units per turn in the disk to the $16\frac{1}{2}$ units per turn of the helix. The trick of winding the RNA snake into the protein basket had the audience totally hypnotized.

A 10 Å map of TMV coat protein was shown by K. C. Holmes (Max-Planck Institute, Heidelberg) and some good looking diffraction data on adenovirus ($6 \times 60,000$) were reported by R. M. Franklin (Public Health Research Institute, New York). The octahedral symmetry of satellite tobacco necrosis virus, which was beautifully presented by B. Strandberg (University of Uppsala) on the basis of the symmetry of the X-ray diffraction patterns, was greeted with shocked animosity especially by the Cambridge workers. The discussion centred on whether holes contributed more or less than matter to the diffraction patterns, and whether the use of rotation functions was justified. A cooperative resolution of this problem is awaited with interest.

During the meeting many of the crystallographers cautioned against speculative discussion based on low resolution maps. D. C. Phillips (University of Oxford) took up this point in his closing remarks, noting that detailed refinement of a protein backbone shows that peptide bond angles may be as much as 25° from the hitherto presumed 180° . Perhaps the time is coming when even a 2.0 Å resolution structure is not a sound enough base from which to interpret the details of protein function, and protein crystallographers may be forced to higher resolution in particular cases. In closing, Phillips remembered, as had successive chairmen, how great had been the contribution of men such as Bernal, the Braggs and Pauling. The mass of new data presented at this meeting and the prospect of at least ten new protein structures per year cry for minds of similar calibre to search for underlying principles.

Late Pre-Cambrian Glaciation in Australia as a Stratigraphic Boundary

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The widespread occurrence of Late Pre-Cambrian glaciogenic sedimentary rocks provides an ideal situation for the establishment of a worldwide Pre-Cambrian chronostratigraphic unit. In this article such a unit based on the well exposed and well documented Late Pre-Cambrian glaciogenic rocks is proposed for use in Australia. It is hoped that the unit will eventually be accepted for use throughout the world.

THE Pre-Cambrian covers 85% of the total length of geological time and is the longest geological division. But although numerous regional and continent-wide schemes exist for its chronostratigraphic and geochronologic subdivision, there is still no generally accepted global subdivision into sections of system (period) and higher rank.

The establishment of global subdivisions for the Upper (Late) Pre-Cambrian¹, or the Proterozoic, is particularly important. We think that much progress can be made by comparing well preserved Late Pre-Cambrian sequences of sedimentary rocks in the northern and the southern hemispheres for the establishment of stratotypes. Late Pre-Cambrian sedimentary rocks that underlie Early Cambrian sequences without a significant stratigraphic break form a natural starting point for stratigraphic studies in the Pre-Cambrian. All stratigraphic methods of correlation, both biostratigraphic and physical, that are used for Phanerozoic rocks should be extended as far as possible in the Pre-Cambrian part of the stratigraphic column.

We propose the use of the Late Pre-Cambrian glaciation in Australia as a stratigraphic boundary to define a continent-wide unit extending from the start of the Sturtian Glaciation in the Adelaide Geosyncline and of the Moonlight Valley Glaciation in the East Kimberley region in Western Australia to the beginning of the Cambrian. Late Pre-Cambrian strata in many parts of Australia contain evidence of a long and uncommonly severe ice age towards the end of Pre-Cambrian (Adelaidean) time. A large part of Australia is known to have been glaciated about 750–670 m.y. ago. The ice age consisted of at least two major glaciations and left immense deposits of relatively unmetamorphosed glaciogenic sediments and other evidence, including striated pavements, as a record

of a great ice age. The pertinent sedimentary sequence connected with the ice age extends from preglacial through various glacial and interglacial sediments to postglacial sediments. The Late Adelaidean sedimentary sequences in the East Kimberley region and in South Australia contain some of the best exposed, most extensive and least disturbed Pre-Cambrian glaciogenic rocks in the world².

Glaciations Outside Australia

Several glaciations have been reported in the Pre-Cambrian. The oldest, the Gowganda Glaciation, took place in southern Canada about 2,200 m.y. ago. The most widespread glaciation is represented by glaciogenic sedimentary rocks, including tillites, with an assumed age of about 600 m.y., which occur in the British Isles, Scandinavia, Spitsbergen and Greenland in the North Atlantic area. Similar rocks of assumedly the same age occur in many parts of Africa and in north-eastern Asia, and, possibly, in South America.

Mawson³ collected and discussed the evidence of Late Pre-Cambrian glaciations, listing tillites and tilloids from all over the world. He concluded that the available evidence proved the existence of a worldwide and severe ice age. Cahen⁴ and Harland^{5,6} also collected evidence of Late Pre-Cambrian glaciations. Harland found evidence for an ice age consisting of two or three severe and prolonged main glaciations. Saito⁷ listed Late Pre-Cambrian tillites and discussed the global extent of the glaciations with particular reference to north-eastern Asia, northern Europe and Arctic regions.

The Late Pre-Cambrian ice age seems to have been longer and of a more severe climate than any of the Phanerozoic glaciations. The most widespread among the Late Pre-Cambrian glaciations is probably the oldest, characterized by a double tillite horizon in several parts of the world. The Late Pre-Cambrian glaciation ranks as one of the outstanding and exceptional physical events in the history of the Earth. In Australia, it was definitely long, covering a time span of approximately 100 m.y. Therefore, it is to be expected that different ages will be obtained for Late Pre-Cambrian glaciogenic rocks in different parts of the world, and that the glacial periods, or the maxima of glaciation, in the northern and the southern hemispheres were not necessarily synchronous^{5,6,8}.

In the northern hemisphere, the Late Pre-Cambrian glaciation has been variously called the Infracambrian or Eocambrian Glaciation or the Varangian (Varegian, Varanger) Ice Age. Because the Subcommission on Pre-Cambrian Stratigraphy of the International Union of Geological Sciences concluded, in 1969, that the names Eocambrian, Infracambrian (and Subcambrian) should be dropped¹, we prefer to use the name Late Pre-Cambrian glaciation. We are not in

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favour of the name, the Varangian (and so on) Ice Age because this glaciation so far has not been satisfactorily dated.

We have searched the literature for reliable radiometric ages for Late Pre-Cambrian glaciogenic rocks, but they seem to be rare. In the northern hemisphere, the only adequately dated possibly glaciogenic rocks seem to be three tillites or tilloids from the USSR, with ages of 715, 810 and 810–747 m.y., given in Salop⁹. These ages are K–Ar ages obtained on glauconites. No radiometric age data are available for the Late Pre-Cambrian tillites in Greenland (David Bridgwater, personal communication). The age data for Australian Late Pre-Cambrian glaciogenic and related rocks are shown in Fig. 1.

Saito⁷ listed four glaciations in the Late Pre-Cambrian and one in the Early Cambrian of China, namely, the Huishan Glaciation 950 m.y. ago, the Hsiho Glaciation 750 m.y. ago, the Wuhsingshan Glaciation 650 m.y. ago, the Nantou Glaciation 600 m.y. ago, and the Cambrian Glaciation 570 m.y. ago. These ages need to be confirmed, however.

The Sturtian (Moonlight Valley) and the Marinoan (Egan) Glaciations recognized in Australia have also been registered elsewhere in the southern hemisphere, namely in southern and central Africa. Roberts¹⁰ made an attempt to correlate Late Pre-Cambrian (Adelaidean) sequences in southern Africa and Australia, assuming the contemporaneity of tillites. Unfortunately, no age data are available for the several Late Pre-Cambrian tillites in South Africa except the information that they occur in a succession of rocks whose sedimentation began about 900 m.y. ago (John de Villiers, personal communication).

Late Pre-Cambrian Glaciation

Evidence of Late Pre-Cambrian (Adelaidean) glaciation is widespread throughout the central part of Australia from the

Kimberleys in the north probably to King Island in Tasmania in the south (Fig. 2).

The principal evidence is the presence of glaciogenic sedimentary rocks—mostly containing faceted, striated and polished boulders—and glaciated pavements in the Kimberley region and in the Adelaide Geosyncline (Fig. 2).

Two separate periods of glaciation are represented. The older glaciation, called the Sturtian¹¹ or the Moonlight Valley² Glaciation, is the most widespread and is commonly represented by two tillite units. The younger glaciation (the Marinoan or the Egan Glaciation) has generally produced thinner and more localized tillites than has the older glaciation.

A feature that many tillite exposures have in common is the presence of a thin pink laminated dolomite unit which crops out immediately above the tillite (Fig. 1). The dolomite appears above the lower tillites in the Kimberley region and above the upper tillite in the central Australian basins in the Adelaide Geosyncline and in the Broken Hill area. Its significance is not known, but a similar dolomite has been noted in the Congo-Zambia region of Africa¹⁰, and dolomite or limestone is a common associate of the Late Pre-Cambrian tillites in the northern hemisphere^{12,13}.

Radiometric age determinations have been carried out on shales within, above and below the tillites in the Kimberleys¹⁴, the Amadeus Basin^{15,16}, and the Adelaide Geosyncline¹⁷. The most definitive of these ages were obtained from the Kimberleys where numerous samples from different stratigraphic levels were dated. The age determinations from other areas were carried out on individual samples or very small groups of samples, and, although in general agreement with the Kimberley ages, cannot be given much individual significance in dating the glaciations.

In several places a sedimentary sequence with probably only minor interruptions passes up from the uppermost glaciogenic

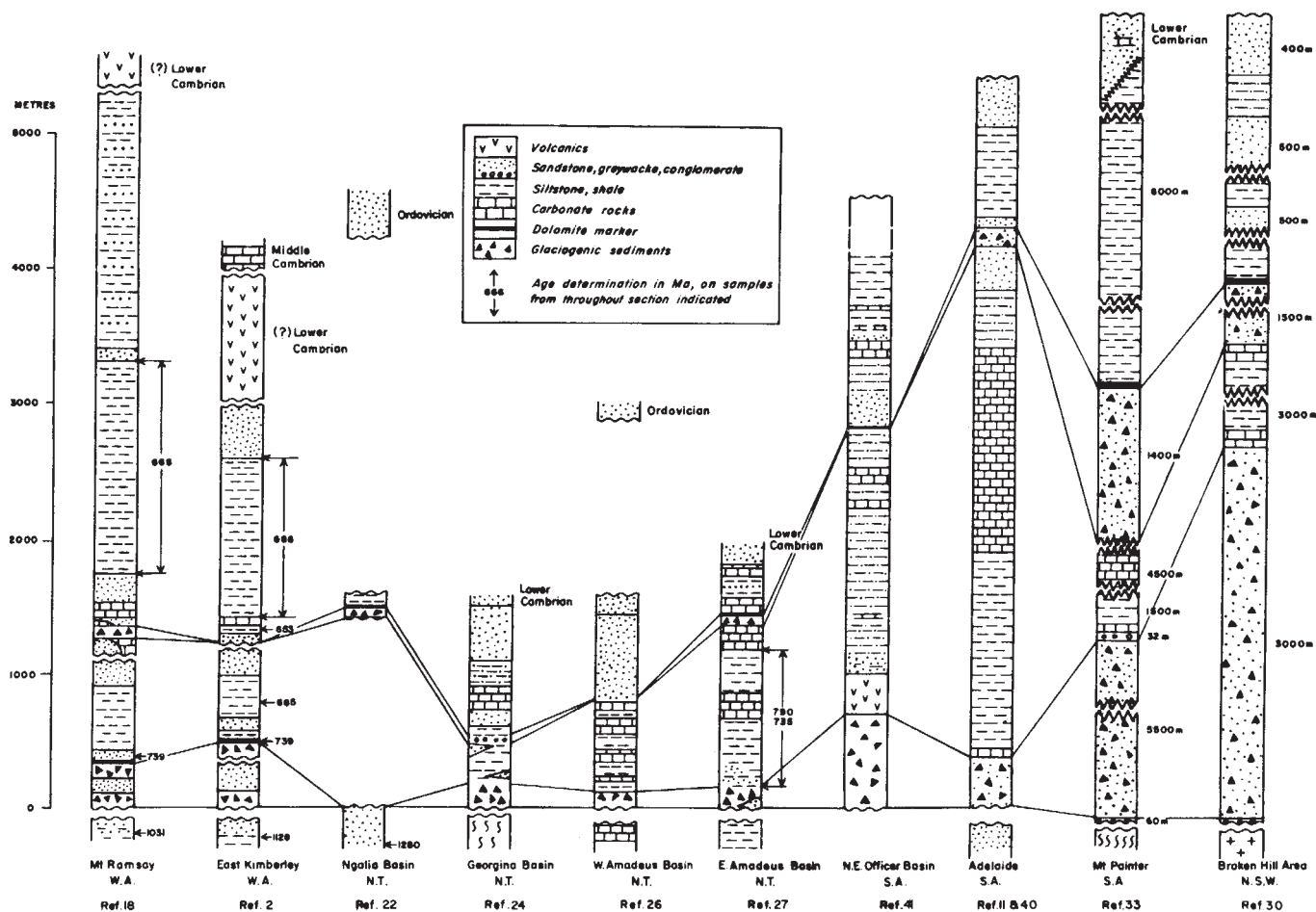


Fig. 1 Stratigraphic and age data for Late Pre-Cambrian glaciogenic sedimentary rocks and related rocks in Australia.

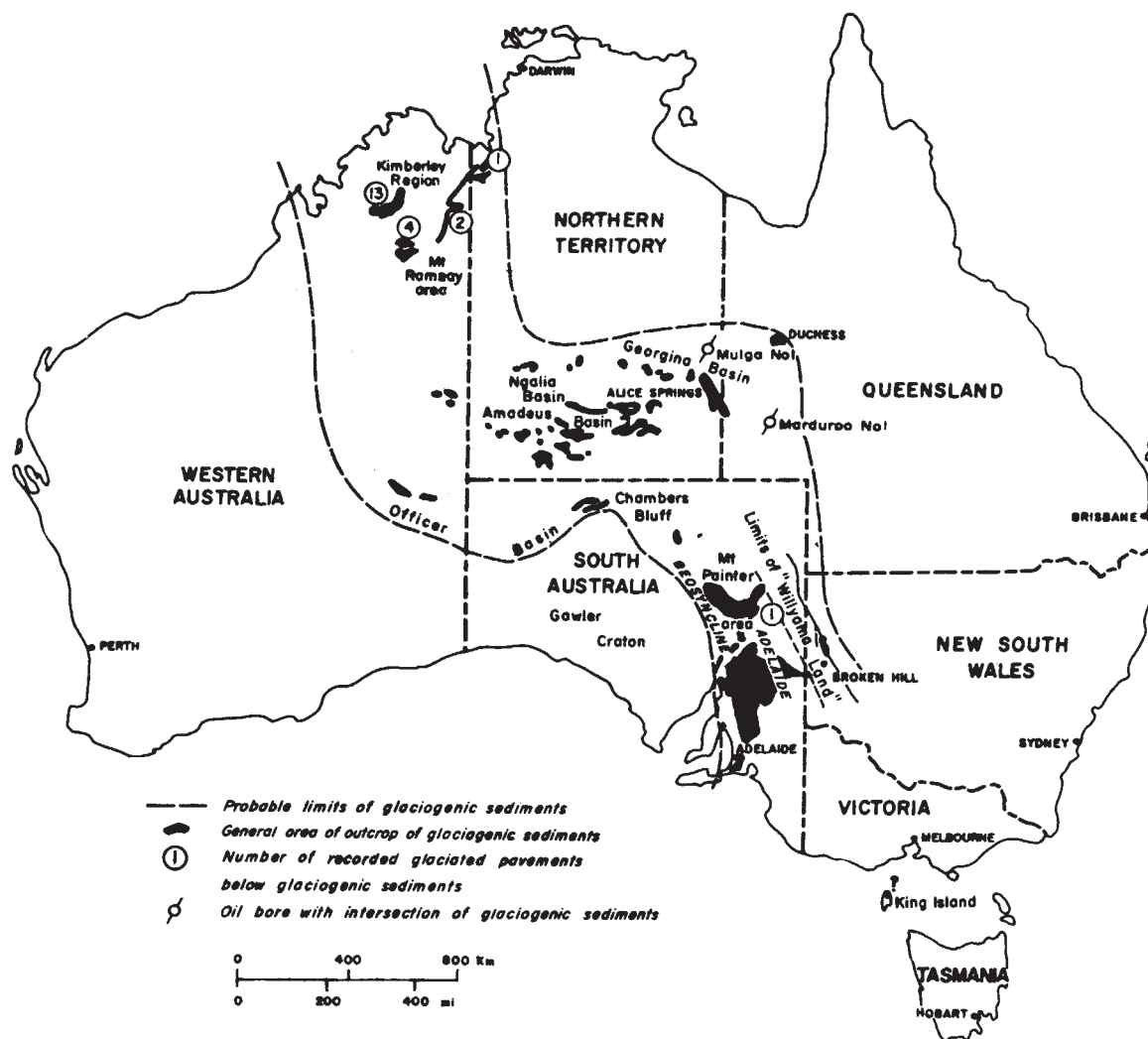


Fig. 2 Distribution of Late Pre-Cambrian glaciogenic sedimentary rocks and glaciated pavements in Australia.

strata into fossiliferous Cambrian rocks. The thickness of this sequence ranges from about 300 m in the Amadeus Basin to about 6,000 m in the rapidly subsiding trough of the Adelaide Geosyncline. The Kimberley glaciogenic sequences all have unconformities between them and the Cambrian rocks.

The tillites in the Kimberley region^{2,18} are noteworthy for the prevalence of striated, faceted and polished boulders, the remarkably fresh and unmetamorphosed state of the matrix and the presence of underlying glaciated pavements. Throughout the Kimberley outcrops, the first period of glaciation is mostly represented by two tillite units separated by a sandstone. The second glaciation is represented only in the southerly (Mount Ramsay) part. The upper of the two lower tillites is overlain by the thin flaggy pink dolomite marker. Bofinger¹⁴ (also quoted in ref. 2) has carried out extensive radiometric dating on illitic sedimentary rocks underlying and overlying the tillites; he produced eight separate total-rock Rb-Sr isochrons from seventy-two samples. From his results we conclude that the lower tillites are about 750 m.y. old and that the upper tillite is about 670 m.y. old (Fig. 1). The upper and lower tillites are separated by about 1,000 m of chiefly shale and siltstone and an unconformity, and the upper tillite is overlain by about 4,000 m of chiefly shale followed unconformably by Lower Cambrian volcanics.

Glaciated pavements are most common beneath the lower tillites¹⁸⁻²¹, but have also been noted beneath the upper tillite¹⁸. Most of the pavements show that the ice sheet moved from north to south, although pavements beneath the lowest

of the older tillites show local variations in the direction of the movement.

In the Ngalia Basin in Northern Territory, about 100 m of glaciogenic sedimentary rocks contain some faceted and striated, but chiefly rounded, boulders²². The glaciogenic sedimentary rocks are underlain by siltstone and dolomite which unconformably overlie sandstone in which glauconites have been dated at about 1,280 m.y.²³. They are overlain in places by laminated dolomite which is unconformably overlain by Ordovician rocks. Wells *et al.*²² correlated these glaciogenic rocks with the upper tillite unit found elsewhere.

In the Georgina Basin, several units which contain striated, polished and faceted boulders in a clay matrix were mapped below fossiliferous Lower Cambrian rocks by Smith²⁴, who correlated some of these boulder beds on lithological grounds with the lower tillite of the Amadeus Basin and of the Adelaide Geosyncline, and some with the upper tillite, but no one section contains both tillites. De Keyser²⁵ has noted glaciogenic rocks at Duchess in Queensland, and they have been recognized in Mulga No. 1 and Mardurroo No. 1 drill holes, thus showing that the glaciation extended from Northern Territory well into Queensland. Generally, the section overlying the glaciogenic rocks and underlying fossiliferous Cambrian sedimentary rocks is less than 100 m thick.

Boulder beds with faceted and striated boulders^{26,27} occur in a number of sections in the Pre-Cambrian succession of the Amadeus Basin, but only two occur in any one section. The oldest again is the most widespread and is associated with

sedimentary rocks, including carbonate rocks, which show rapid lateral variation. The younger boulder beds are confined to the north-eastern part of the basin and are overlain by a laminated pink dolomite marker. The two boulder beds are separated by about 1,200 m of shale and limestone, and the upper bed is overlain by a further 300 m of sandstone, shale and dolomite beneath conformable fossiliferous Lower Cambrian sedimentary rocks. The shale yielded ages of 790 m.y.¹⁵ and 735 ± 45 m.y.¹⁶. The older age is based on the mean of two Rb/Sr isochrons, one from two samples and the other from three samples. The younger age is from an isochron on sixteen samples including the samples used by Bofinger.

The Sturtian and Marinoan glaciogenic and interglacial sedimentary rocks extend over the Adelaide Geosyncline and north-westwards into Western Australia. Mapping evidence and study of erratics show conclusively that the Sturtian Glaciation eroded a large belt of crystalline basement ("Willyama Land"), presumably ice-capped, along the eastern flank of the Adelaide Geosyncline from the Broken Hill region in New South Wales to Mount Painter in South Australia, where a glaciated pavement has been observed. The basement probably extended northwards about 1,400 km, from Adelaide to the Georgina Basin in Northern Territory. Although no pavements have been discovered on the western side of the geosyncline, numerous striated and faceted erratics of western provenance have been identified.

In the Officer Basin, a tillite with abundant faceted and striated erratics of Adelaidean and basement rocks occurs in the Chambers Bluff area about 1,000 km north-west of Adelaide. Daniels²⁸ described a similar tillite about 650 km farther west in Western Australia.

The prolongation of the glaciogenic belt south-east of Adelaide is conjectural owing to the superposition of a wide and intense north-easterly trending Palaeozoic fold belt.

The Late Pre-Cambrian sedimentary units in the Adelaide Geosyncline are thicker than elsewhere in Australia. In the type area near Adelaide, the Sturt Tillite has a thickness of about 300 m. In the Mount Painter area, the tillite and the glaciomarine sedimentary rocks of the Sturtian glaciation have a thickness of about 5,500 m.

About 320 km north-north-east of Adelaide, tillite changes across fault zones into glaciomarine sequences exceeding 3,300 m thick. Sturtian glaciogenic sequences were deposited in a similar environment on the eastern side of the tectonically active "Willyama Land" in New South Wales, and there have a maximum thickness exceeding 2,000 m.

The Sturtian Glaciation was followed in the Adelaide Geosyncline by the deposition of a dolomite-siltstone sequence about 3,000–5,000 m thick. The dolomite-siltstone facies is very widespread and persistent in Australia and apparently registers an abrupt climatic change at the close of the Sturtian Glaciation.

According to Glaessner, Preiss and Walter²⁹, stromatolites in the interglacial sequence may be correlated with Russian genera and species which occur in the 950–570 m.y. age range.

The effects of the Marinoan Glaciation in southern Australia, as in central and northern Australia, are less intense and widespread than those of the Sturtian Glaciation. The Marinoan glaciogenic sedimentary sequence in the eastern part of the geosyncline is up to 1,500 m thick, and the maximum observed tillite thickness is about 200 m. The tillite contains faceted and striated erratics. In New South Wales, a similar sequence is present in the Broken Hill area.

The Marinoan postglacial sequence is remarkably consistent in facies in its lower part throughout Australia. In the Adelaide Geosyncline, the sequence commenced with the deposition of dolomite, grading upwards into shale. The overlying units of the sequence are siltstones and sandstones with recurrent dolomite. In the western part of the Geosyncline the units are largely red. The maximum known thickness of the sequence in the northern Flinders Ranges is about 6,500 m. A comparable thickness of the possible equivalent units is known in New South Wales³⁰. To the west and south of the Geosyncline, the

thickness of the postglacial sequences declines markedly. The uppermost unit of the sequence, the Pound Quartzite, is the host for the Ediacara fauna, comprising megafossils of a variety of soft-bodied Pre-Cambrian animals which are of considerable biostratigraphic significance³¹. An erosional event before an Early Cambrian marine transgression occurred on the western side of the Geosyncline. It is possible that sedimentation was continuous from the Adelaidean into the Cambrian in the central part of the Geosyncline.

A tillite-like rock occurs on King Island in Tasmania³², apparently a genuine tillite (Emyr Williams, personal communication).

Sturtian Glaciation as Boundary

Harland^{5,6} suggested the creation of a new System within the Pre-Cambrian, starting with the onset of the great "Infracambrian" glaciation and concluding before the recognized Cambrian faunas, to be called the Infracambrian System or the Varangian System. He⁶ thought that glaciogenic sedimentary rocks may be the best basis for the establishment of Pre-Cambrian Systems which would be internationally recognized.

Elaborating on this suggestion, we propose to use the body of rocks accumulated between the deposition of the oldest Sturtian (Moonlight Valley) glaciogenic rocks in Australia and the deposition of the basal unit of the Cambrian, still to be established, to define a chronostratigraphic unit in Australia. In the Adelaide Geosyncline, the corresponding lithological units are the Umberatana and Wilpena Groups⁴⁰. The Umberatana Group contains two glaciogenic units, the Yudnamutana Subgroup and the younger Yerelina Subgroup. During the past twenty years these units have been mapped in detail over an area of about 250,000 km² in the Adelaide Geosyncline alone and have without a doubt established the chronostratigraphic significance in this area of the Sturtian and Marinoan glaciations. The base of the proposed unit is to be marked by a reference point, called the Sturt Marker (Marker Horizon), provisionally placed in South Australia at the top of the granite conglomerate which forms the bottom member of the Fitton Formation³³. The locality is in the type section in the Mount Painter region on the eastern flank of the Adelaide Geosyncline. The granite conglomerate member probably includes some preglacial lag talus, but the conglomerate grades upwards into a glaciomarine sequence which interfingers with the tillite of the overlying Bolla Bollana Formation³³. The overlying glaciomarine sequence passes upwards into tillite. It is the thickest (5,500 m) known glaciogenic sequence in Australia and is believed to incorporate the earliest sedimentary record of the Sturtian Glaciation. Thomson³⁴ discusses in more detail the stratigraphic aspects of the selected site and the need for redefining the Sturtian Series¹¹.

The corresponding continent-wide geochronologic unit represents the time between the onset of the Sturtian (Moonlight Valley) Glaciation and the beginning of the Cambrian Period (end of the Pre-Cambrian). We propose to refer to it provisionally in Australia as the Late Adelaidean.

We tentatively place the beginning of the Sturtian (Moonlight Valley) Glaciation at about 750 m.y. ago, based on the probable age of the early phase of the Moonlight Valley Glaciation (Table 1). We also tentatively place the beginning of the Cambrian Period, and the end of Pre-Cambrian time, at 570 m.y. ago, pending a more accurate age for the Cambrian-Pre-Cambrian boundary. We propose to place the Sturt Marker in South Australia largely because of the completeness of the sedimentary record and because there may be no significant stratigraphic break between the start of the Sturtian Glaciation and the Cambrian in some parts of that state. Although more accurate age data are available from the Kimberleys, several unconformities are evident there between the glaciogenic sedimentary rocks and the Cambrian strata.

When more radiometric ages for the sedimentary rocks underlying and overlying the tillite units become available, the

beginning of the glaciation can be dated more accurately. The oldest age thereby obtained for the glaciation will be the definitive one.

Table 1 Age Relationships of the Late Pre-Cambrian Glaciations in Australia

		Age, m.y.
Adelaidean Proposed unit	CAMBRIAN	570
	Subunit 1	Egan Glaciation and Marinoan Glaciation
	-----	700
	Subunit 2	Moonlight Valley Glaciation and Sturtian Glaciation
		740 (Late phase) 750 (Early phase)
		750 Sturt Marker

The proposed geochronologic unit has a time span of about 180 m.y. or somewhat longer than the duration of the Mesozoic Era. As to giving a formal name to the proposed chronostratigraphic unit, at this stage we prefer only to define its lower boundary by the Sturt Marker reference point. We would, however, prefer to use a name totally unconnected with geographic names and names pertaining to Earth history.

There is nothing remarkable in placing a stratigraphic boundary at the beginning of a glaciation. This has been done previously, when the boundary between the Pliocene and the Pleistocene—or between the Tertiary and the Quaternary—was placed at the abrupt and severe onset of the first Pleistocene glaciation. Global glaciations are major geological events and mark the beginning of episodes of cold-facies sedimentation. Thus they are “natural boundaries” or “natural breaks” indicating important global geological events^{35,36} and are inextricably tied to the rock record. We think that dating a glaciation produces more definite and accurate information on the age of a geological event than can be obtained by dating an orogeny with a hazy beginning and a vague end.

Problems Remaining

In our proposal to designate a chronostratigraphic unit for the Upper Pre-Cambrian in Australia, we have followed suggestions made by McDougall *et al.*³⁷, Dunn *et al.*³⁸, and Crook³⁹ relative to defining chronostratigraphic and geochronologic units. The use of the chronostratigraphic unit, and of the corresponding geochronologic unit, in Australia is suggested. We repeat that, actually, we have defined only the lower boundary of the chronostratigraphic unit, because no consensus exists of the position of its upper boundary (the Cambrian–Pre-Cambrian boundary). We hope that, when the age of the lower boundary of the unit has been established accurately by radiometric dating, and when the problem of the Cambrian–Pre-Cambrian boundary has been settled, the corresponding geochronologic unit will be found favourable for worldwide correlation and will be accepted as a global unit and used as a standard of reference, whether or not exactly similar sequences may exist elsewhere.

The problem of the rank of the proposed unit still remains unsettled. It is either an Erathem (Era) or a System (Period). Because of the long duration of the Pre-Cambrian we would prefer to consider it a system.

Other Late Pre-Cambrian stratotypes may also be selected in Australia for possible use in global correlation³⁸. The Late Pre-Cambrian in Australia is characterized by a largely continuous succession of unmetamorphosed sedimentary rocks ranging from the Middle Cambrian far down into the Pre-Cambrian. In the Adelaidean and Carpentarian sequences, down to rocks as old as 1,800 m.y., the correlation possibilities offered by stromatolite stratigraphy should not be forgotten²⁹.

Finally, we should like to call the attention of Pre-Cambrian geologists and stratigraphers outside Australia to the unique Australian Late Pre-Cambrian sequences of sedimentary rocks, hoping to promote their comparison and correlation with corresponding sequences in other parts of the world.

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Relationship of Centromeric Heterochromatin to Fluorescent Banding Patterns of Metaphase Chromosomes in the Mouse

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Human and mouse chromosomes can be distinguished on the basis of different amounts of centric heterochromatin and different patterns of fluorescent banding.

Two recent observations have major implications for cytogenetics. The first is the specific localization of mouse satellite DNA primarily near the centromere of mouse chromosomes with the use of *in situ* hybridization^{1,2}. Mouse satellite DNA, comprising about 10% of the total nuclear DNA, consists of highly repetitive nucleotide sequences which therefore reanneal relatively rapidly after denaturation with alkali³. Yasmineh

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and Yunis⁴ have suggested that satellite DNA is a major component (70%) of heterochromatin. Centromere regions presumed to be rapidly reannealing DNA stain intensely with Giemsa after alkali denaturation and heat renaturation. This material has been called centromeric or centric heterochromatin⁵ and is considered to be constitutive heterochromatin⁵. The second observation is the presence in chromosomes of humans^{6,7}, primates⁸, and probably other mammalian species of specific fluorescent banding patterns demonstrated with quinacrine derivatives which are unique and reproducible and thus permit identification of many of the chromosomes. We have found similar specific fluorescent banding patterns in a mouse tissue culture cell line (Fig. 1a).

A relationship between centric heterochromatin and fluorescent banding patterns became apparent when these two techniques were combined to identify individual mouse chromosomes in a cell line (I-R) which is an 8-azaguanine resistant derivative of an L cell line⁹. The modal chromosome

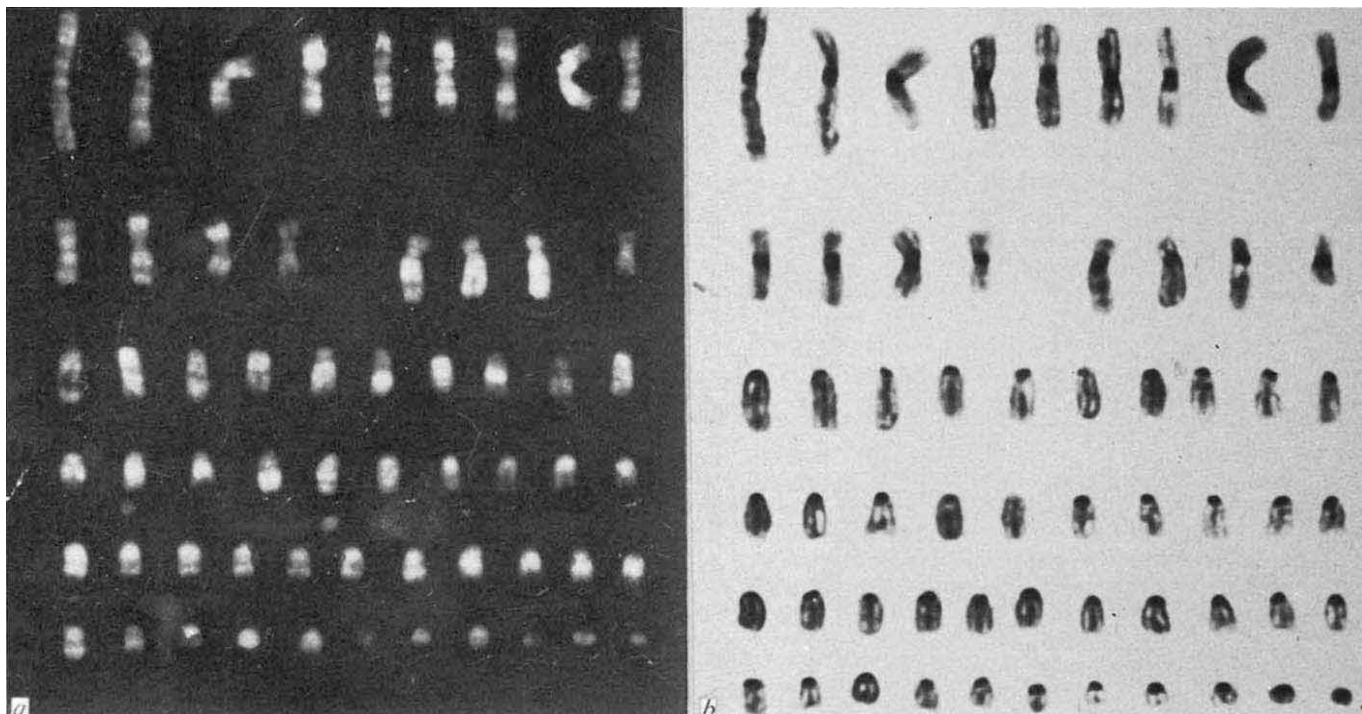


Fig. 1 *a*, Karyotype of metaphase chromosomes from mouse I-R cell line, stained with quinacrine dihydrochloride and photographed with ultraviolet fluorescence. There are 59 chromosomes, 17 of which are banded and 42 of which have terminal centromeres. Most of the chromosomes appear to be distinguishable on the basis of their banding patterns. The symmetrical banding pattern, especially clear in the sixth chromosome, line 1, and the first and third chromosomes in line 2, suggests that these might be isochromosomes. The smallest banded chromosome (end of line 2) is not usually present in the I-R karyotype. Groups of up to four acrocentric chromosomes of similar size with similar banding patterns (for example, Nos. 2-5, line 5) suggest that they may be homologues originally derived from a tetraploid or pseudo-tetraploid cell. *b*, Karyotype of same cell as in (*a*) after alkali denaturation, heat renaturation and Giemsa staining. Intensely staining centromeric regions are present in all banded chromosomes. The largest chromosome also has three areas of staining in the short arm and four such areas in the long arm. The variation in the size of centric staining is clearly evident. The fifth chromosome, line 4, clearly does not show centric staining.

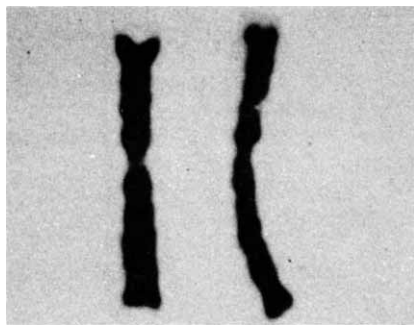


Fig. 2 The long banded chromosome of I-R stained with orcein from two different cells has, in addition to the centromere constriction, three sites of decreased staining and narrowing of the chromosome in the short arm and four sites in the long arm, which correspond to the fluorescence and Giemsa banding patterns. These are more prominent in the less contracted chromosome on the right.

number of I-R is 58 and the large number of banded chromosomes (18) reflects the many chromosomal rearrangements that have occurred in culture. The normal mouse karyotype consists of 40 acrocentric chromosomes. The largest banded chromosome in I-R is unique and is readily identifiable in different metaphase plates.

Chromosomal preparations of the I-R cell line were treated in three ways. First, some preparations were stained with aceto-orcein and areas of reduced staining, which presumably represented sites of secondary constrictions¹⁰, were noted. Other slides were stained initially with quinacrine dihydrochloride and suitable metaphase cells were photographed following the procedure of Pearson *et al.*¹¹. The same chromosome preparations were treated with HCl, RNAase and NaOH, the DNA was allowed to reanneal at 60° C for 18 h and the cells were stained with Giemsa, according to the technique of Gall and Pardue¹², except that subbing of slides was omitted.

Photographic negatives of metaphase chromosomes stained with aceto-orcein, quinacrine dihydrochloride, or Giemsa were scanned with a Joyce-Loebl microdensitometer and a tracing of the density of the chromosome was recorded.

In orcein stained slides, the large banded chromosome of I-R showed a constriction at the centromere as well as three sites of reduced staining and chromosomal narrowing in the short arm and four such sites in the long arm (Fig. 2). Several other banded chromosomes showed very prominent constrictions in the centromeric region.

Twenty-five I-R cells in metaphase, stained with quinacrine dihydrochloride, and photographed with fluorescence demonstrated that all the mouse chromosomes have fluorescent bands (Fig. 1a). The large banded chromosome consistently showed multiple bands with reduced fluorescence of varying width. The centromere region generally showed the least fluorescence of any portion of the chromosome. The short arm generally contained three bands of decreased fluorescence with the band near the centromere being most prominent, that nearest the end being intermediate and that in the middle of the short arm being quite faint and not distinguishable in some cells. The long arm contained four bands of which that near the centromere was most distinct while the three bands relatively evenly spaced along the long arm showed less loss of fluorescence. In some cells, particularly those later in metaphase, the middle bands were less readily visible.

The other banded chromosomes showed distinctive patterns of increased and decreased fluorescence which allowed the individual identification of most of these chromosomes. Symmetrical banding patterns in some of the metacentric chromosomes suggested that these chromosomes were either isochromosomes or formed by centric fusion of homologous chromosomes. The centromere regions of some of the banded chromosomes were characterized by large areas of diminished

fluorescence. Many of the acrocentric chromosomes could also be identified individually on the basis of the position and intensity of the fluorescent bands.

In the preparations stained with Giemsa, the same twenty-five cells were re-examined to determine the distribution of rapidly reannealing DNA (Fig. 1b). The large banded chromosome had a densely stained centromeric region that we presume to be centric (constitutive) heterochromatin. In some late prophase chromosomes, this centromere region seemed to consist of two dense masses separated by a narrow, lightly stained area. The short arm contained three distinct Giemsa staining bands with the one nearest the centromere the most intense and the one in the middle the least intense. The long arm had four such bands with that nearest the centromere most intense and the third one from the centromere least densely stained. The edge of all the chromosomes was usually rather densely stained, but was not included as a band. The densely stained heterochromatin in the other banded chromosome was all located near the centromere and the amount seemed to vary from one chromosome to another. The acrocentric chromosomes, with two or three fairly consistent exceptions, also had varying amounts of centric heterochromatin. The presence of centric heterochromatin in the chromosomes stained with Giemsa was very helpful in orienting some of the fluorescent banding patterns, particularly in the small acrocentric chromosomes. The heterochromatic areas of these chromosomes stained first with quinacrine and then treated to reveal rapidly reannealing DNA appear smaller and stain less intensely than these same areas in chromosomes without prior exposure to quinacrine (Fig. 3).

Microdensitometric tracings of the chromosomes reflect the variations in staining intensity already noted with orcein, quinacrine and Giemsa. Three tracings for the large banded chromosome stained with quinacrine and restained with Giemsa are reproduced in Fig. 4. It is readily apparent from the examination of this chromosome that the areas of diminished fluorescence correspond precisely to those of centric heterochromatin. Although the other banded chromosomes showed specific banding patterns of fluorescence, none of them demonstrated dense bands of Giemsa staining except for the centromeric region. Those chromosomes which on fluorescence had exceptional dullness in the region of the centromere had the largest amount of centric heterochromatin.

It thus becomes apparent that there is a direct correlation between the site of centric heterochromatin, identified both as a secondary constriction and a densely Giemsa staining area, and the location of diminished fluorescence. Further, within the limits of the observations, the amount of centric heterochromatin seems to be proportional to the loss of fluorescence from a particular portion of the chromosome. These results are not unexpected, if, as Caspersson¹³ has proposed, quinacrine mustard (and possibly the quinacrine dihydrochloride



Fig. 3 Long banded chromosomes from two different cells showing that the intensely Giemsa stained regions are larger and more distinct in chromosomes that have not had prior staining with quinacrine.

used in this investigation) binds to guanine. Mouse satellite DNA has a relatively low G-C content¹⁴ and so one might expect, on this basis, that the satellite-containing centromeric regions should demonstrate decreased fluorescence, which they do. The absence of dense Giemsa bands in other chromosomes which show areas of decreased fluorescence may reflect a relatively lower guanine content of these chromosome regions but would also be due to relatively fewer repetitive nucleotide sequences on the assumption that repetitive sequences are the primary differentiating factor for this method of staining.

These observations on mouse chromosomes may have

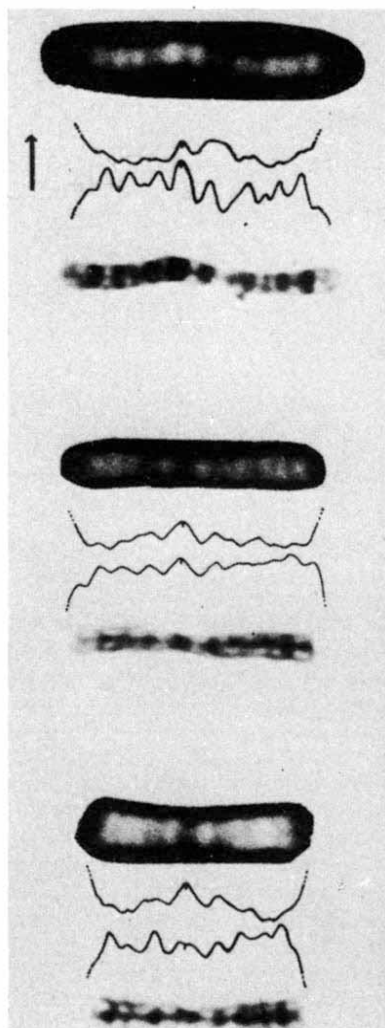


Fig. 4 The long biarmed chromosome of I-R which has been stained with quinacrine (upper) and Giemsa (lower) from three different cells. In each case the short arm is to the left of the centromere and the long arm to the right. The microdensitometric tracing of light transmission through the photographic negative from each chromosome is adjacent to the corresponding chromosome. The arrow indicates the direction of increasing transmission through the negative. The centromere regions of chromosomes and their tracings are aligned precisely, to show the correspondence of the Giemsa and quinacrine banding patterns. The degree of chromosome contraction increases from top to bottom. The apparent fusion of some of the intermediate bands in the short and long arm can be seen in the more condensed chromosome. The upper tracing in each pair represents the light transmission through the negative photographed with ultraviolet fluorescence. In these, the areas of decreased fluorescence, for example at the centromere, permit the largest amount of light to pass through and therefore give rise to a peak in the tracing. Since the background area is light on the negative, the edge of the chromosome is represented as a rise in the curve. The lower tracing in each pair represents the light transmission through the negative, photographed with bright light microscopy. On the negative, the centromere is least dense and permits the greatest light transmission and therefore also appears as a peak. The background is dark and therefore the line curves down at the edge of the chromosome.

relevance to human chromosomes even though mouse metaphase chromosomes apparently contain much more centric heterochromatin than do human chromosomes¹⁵. The human chromosomes which were known to have prominent secondary constrictions, that is 1, 9 and 16 (ref. 10), have the largest amounts of centromeric heterochromatin^{15,16} and also show the most prominent decrease in quinacrine fluorescence near the centromere^{6,7}. An apparent exception to this correlation is the human Y chromosome which has a large mass of rapidly reannealing DNA in the distal portion of the long arm¹⁶ but fluoresces very brightly. It is possible that the rapidly reannealing DNA in the Y is repetitive, but has a relatively high G-C content. Alternatively, since bright fluorescence is limited to man, the gorilla and the chimpanzee⁸, its expression may be unrelated to the phenomena under discussion. Though it seems reasonable to assume that the Giemsa stained regions, following alkaline denaturation and renaturation, do reflect highly repetitive nucleotide sequences, the mechanism of the staining is not clear. Giemsa is not known to bind differentially to double stranded DNA. It may be that the single stranded regions are more readily degraded on prolonged incubation than are the double stranded regions, which might account for the rather long period of incubation needed to develop the differential Giemsa staining of the centric heterochromatin.

The fact that human and mouse chromosomes can be distinguished on the basis of (a) different amounts of centric heterochromatin and (b) different fluorescent banding patterns is obviously of great use for the cytogenetic analysis of human-mouse somatic cell hybrids. Centric heterochromatin can be used to identify many of the human chromosomes, while quinacrine fluorescence banding patterns can identify all of them. Similarly, centric heterochromatin can identify some of the mouse chromosomes¹⁵, whereas quinacrine fluorescence banding patterns can identify many of them.

In addition to differentiating mouse from human chromosomes in hybrid cells, fluorescence banding patterns will be helpful in following chromosomal evolution in mouse and other cell tissue culture lines, which may provide models for the cytogenetic analysis of tumour progression and, for example, in identifying those mouse chromosomes which may carry genes for repressing malignancy¹⁷.

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Note added in proof. We have found subsequently that, in the absence of HCl and RNAase treatment, differentially stained Giemsa bands appear along the chromosome in the same location as the quinacrine fluorescent bands. These Giemsa bands are distinguished from those discussed here by their position and their less intense Giemsa staining.

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Atomic Positions in Rhombohedral 2-Zinc Insulin Crystals

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Atomic positions in three dimensions have been derived for the two non-identical molecules of insulin in rhombohedral 2-zinc insulin crystals. Interesting correlations appear in their relation to the sequence variations observed so far in natural insulins.

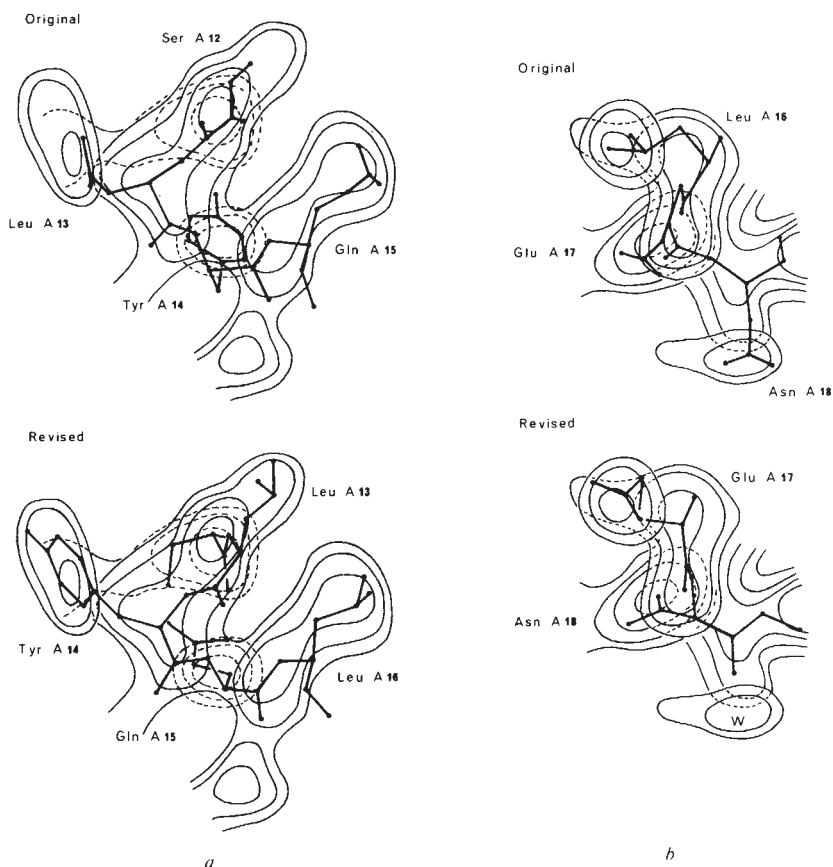
In a previous communication¹ we reported the results of the initial investigation of an electron density map at 2.8 Å

resolution of rhombohedral 2-zinc insulin crystals. We have now analysed this map in more detail and derived coordinates for all the atoms in the two insulin molecules of the crystal asymmetric unit. The essential features of the molecular and crystal structure previously described have remained unchanged, but we have revised our views of the relative arrangement in space of the residues between A 12 and A 18 in the insulin molecules².

Electron Density and the Insulin Model

Our complete atomic coordinates have been reached by a series of approximations. We first built a scale model (2 cm to 1 Å) by direct comparison with the electron density map

Fig. 1 Superimposed electron density contours taken from the electron density map at 2.8 Å resolution in the region of residues A 12–A 18, molecule 2. The drawings show the original and revised interpretations of the atomic arrangement.



of the same scale and set in the optical superposition device constructed by Richards³. The coordinates taken from the map were modified in two alternative ways: (1) by a programme written by Diamond⁴ and designed to impose correct molecular geometry; and (2) by a programme written by M. Levitt (private communication) designed to minimize the energy of interatomic interactions. The programmes produced atomic positions which differed in detail from each other and also occasionally from the map. From them a third, compromise, set of coordinates was derived, in which the atoms were placed by individual model building as far as possible in accordance with both the map and the expected stereochemistry. In most regions, the differences between the alternative atomic positions are quite small (of the order of

0.2–0.3 Å) but there are a few regions in which the uncertainties of residue placing are much greater, particularly B 3 asparagine, B 21 glutamic acid, B 29 lysine and B 30 alanine. These are all involved in intermolecular contacts and may be disordered in the crystal.

Some of the problems of atom placing in relation to the electron density map are illustrated by Fig. 1, which shows the map in the region of A 12–A 18, in molecule 2, with superimposed alternative residue arrangements. In Fig. 1a, the revised arrangement is clearly preferable in terms of the electron density. Moreover, the changes it requires in relation to the molecular structure are highly significant and convincing; both leucines, A 13 and A 16, are turned in towards the central core of the molecule while A 15 glutamine is

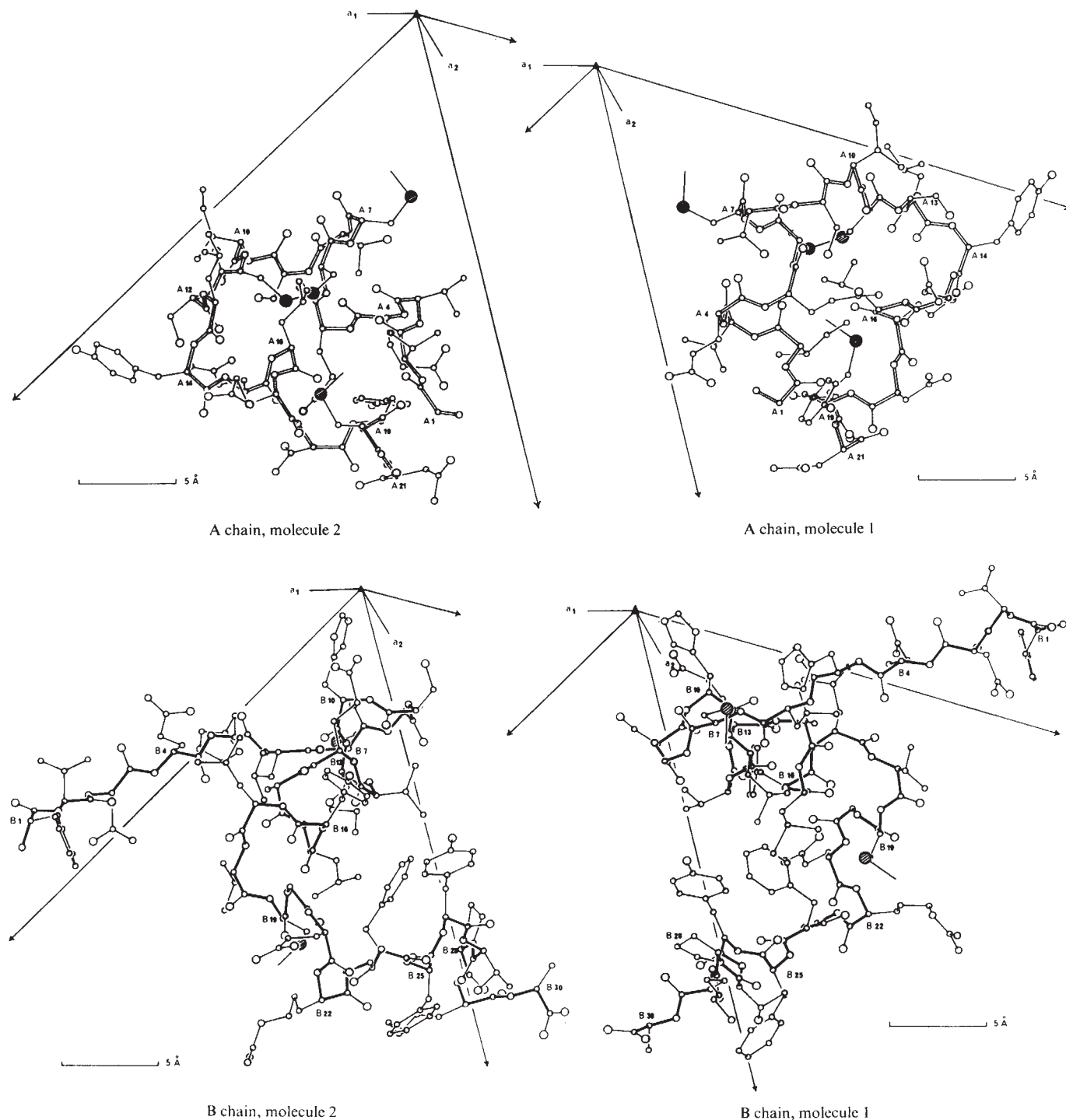


Fig. 2 The atomic positions in the A and B chains of the insulin molecules, 1 and 2, projected along the three-fold c axis. Note that molecule 2 is placed on the left.

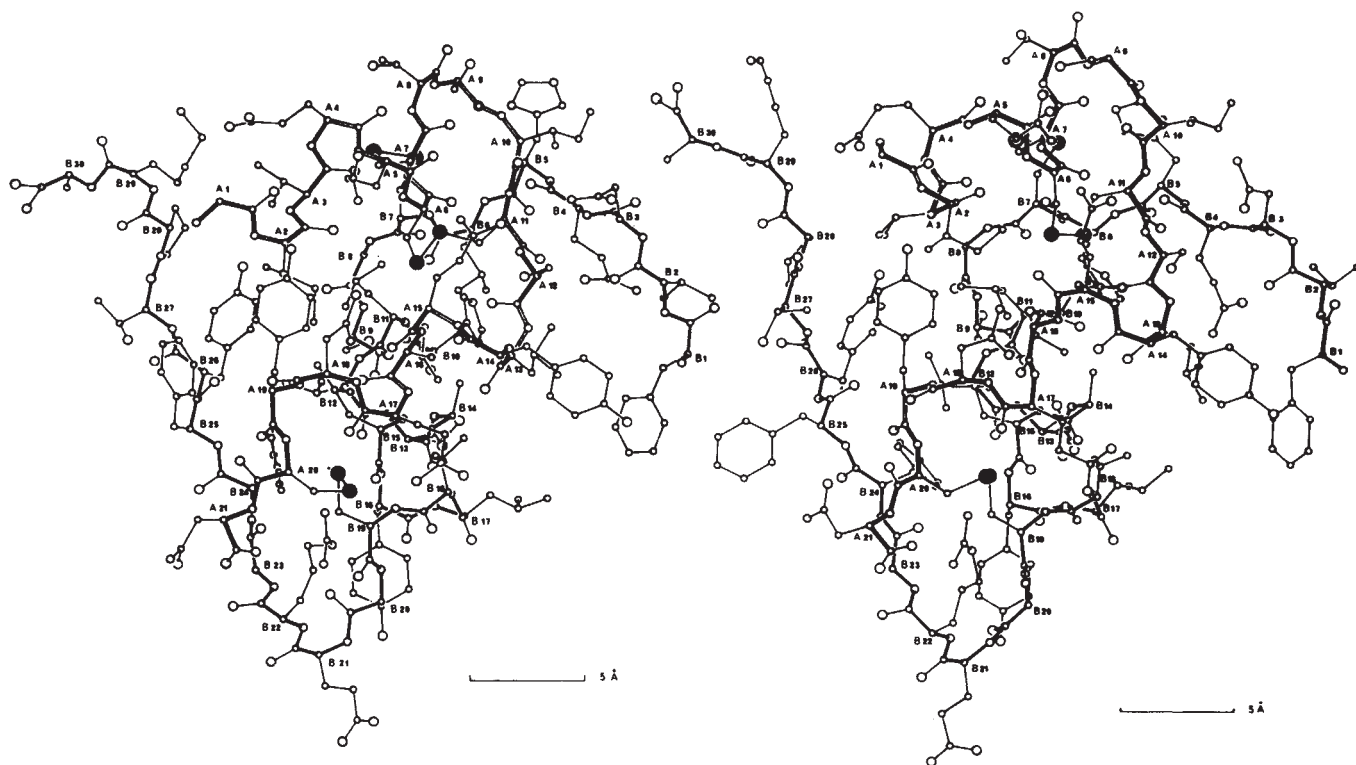


Fig. 3 The atomic positions in the insulin molecules, 1 and 2, in projection in equivalent directions perpendicular to the c axis. Molecule 2 (on left) has been rotated around the two-fold axis (see Fig. 4) so that it can be directly compared with molecule 1.

on the outside, projecting towards the A 5 glutamine; A 14 tyrosine is extended outwards, very readily available for reaction and is hydrogen bonded, hydroxyl to hydroxyl, with A 14 in the neighbouring molecule. In Fig. 1*b* the revised arrangement may look less clearly preferable in terms of the electron density, the peak w being empty. This is now interpreted as occupied by water, hydrogen bonded to the carbonyl group. Detailed study of the map has revealed many similar situations, in which low electron density peaks almost certainly indicate water attached to electro-negative groups. All the

tyrosine residues and most of the carboxyl groups, as well as several other carbonyl groups, have apparent water attachments, often linking the groups concerned to other polar groups.

The Molecular Arrangement

Fig. 2 shows the A and B chains of the two molecules which together constitute the crystal asymmetric unit viewed down the c axis, and Fig. 3 shows the two molecules viewed in equivalent directions perpendicular to the c axis. Although

Table 1 Variations in Insulin Sequences

A Chain																					
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
Gly	Ile	Val Leu	Glu Asp	Gln	Cys	Cys	Thr Ala His	Ser Gly Asn His Arg Lys	Ile Thr Val Pro	Cys	Ser Asp Asn Thr	Leu Lys Ile Arg	Tyr Phe His Asn	Gln Asp	Leu	Glu Gln Met	Asn Ser	Tyr	Cys	Asn	Asp
B Chain																					
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
Phe Ala Tyr	Val Ala Pro	Asn Lys Pro Ala Ser	Gln Arg	His Arg	Leu	Cys	Gly	Ser Pro	His Asn Gln	Leu	Val	Glu Asp	Ala Thr	Leu	Tyr	Leu Ser	Val	Cys	Gly Gln Arg	Glu Asp His	Arg Asp
23	24	25	26	27	28	29	30														
Gly	Phe	Phe Tyr	Tyr Arg	Thr Asn Ile Pro	Pro Ser Asn	Lys — Met Asn	Ala Thr Ser Asp														

See text for further details.

the molecules are clearly constructed on identical principles, there are many detailed differences between them. Some of these are concerned with simple alternative ways of residue packing, such as those involving the two B 25 phenylalanine residues. Others are more obscure in origin. For example, the imidazole rings of the B 10 histidine residues attached to zinc differ in their tilt to the three-fold axis of the crystal. This may, as Brill and Venable⁵ have suggested, be a result of the zinc ions having been associated sequentially with the hexamer formation, so that different geometrical adjustments of the molecules within the hexamer have occurred. The observed differences agree well with the electron spin resonance measurements made on cupric insulin crystals, which indicated two sites not exactly related by two-fold axes for the ligands attached to copper. Apart from these, the most important differences observed between the two molecules are probably those affecting the positions of certain of the A chain residues. The A 19 tyrosine hydroxyl group in molecule 1 seems to be linked through a water molecule to the carbonyl group of A 1 glycine, and in molecule 2, A 19 tyrosine is more closely linked with A 5 glutamine.

Fig. 4 illustrates the way in which the two molecules fit together to form the dimer around an approximately two-fold axis. Fig. 4c shows some details of this arrangement, particu-

larly the formation of hydrogen bonds in an anti-parallel pleated sheet between the B 24 and B 26 peptide groups of the two molecules. As Fig. 5 shows, the three dimers fit together to form a hexamer which is very smoothly spheroidal over much of its surface. The upper and lower faces of the spheroid are, however, penetrated by deep grooves or pockets, formed between the residues of the A chain loop which project from its body. These pockets are filled in the crystal partly by water and partly by the projecting A chain residues of the succeeding molecules, along the direction of the three-fold axis.

Sequence Variation and the Insulin Structure

The interpretation of the electron density map yields some very interesting correlations between the variations of sequence in different natural insulins and the three dimensional structure. Table 1 illustrates the sequence variations observed so far⁶ those in the coypu and guinea-pig, which are rather different from most other creatures, are shown in *italics*⁷.

The invariant residues, the distribution of which in the structure is shown in Fig. 6, fall into several groups. First, there is a group of essentially non-polar residues; the three cystine groups, A 16, B 6, B 11 and B 15 leucines, A 2 isoleucine, B 18 valine and B 8 and B 23 glycines, these largely compose the core of the molecule and ensure the correct relative arrangement

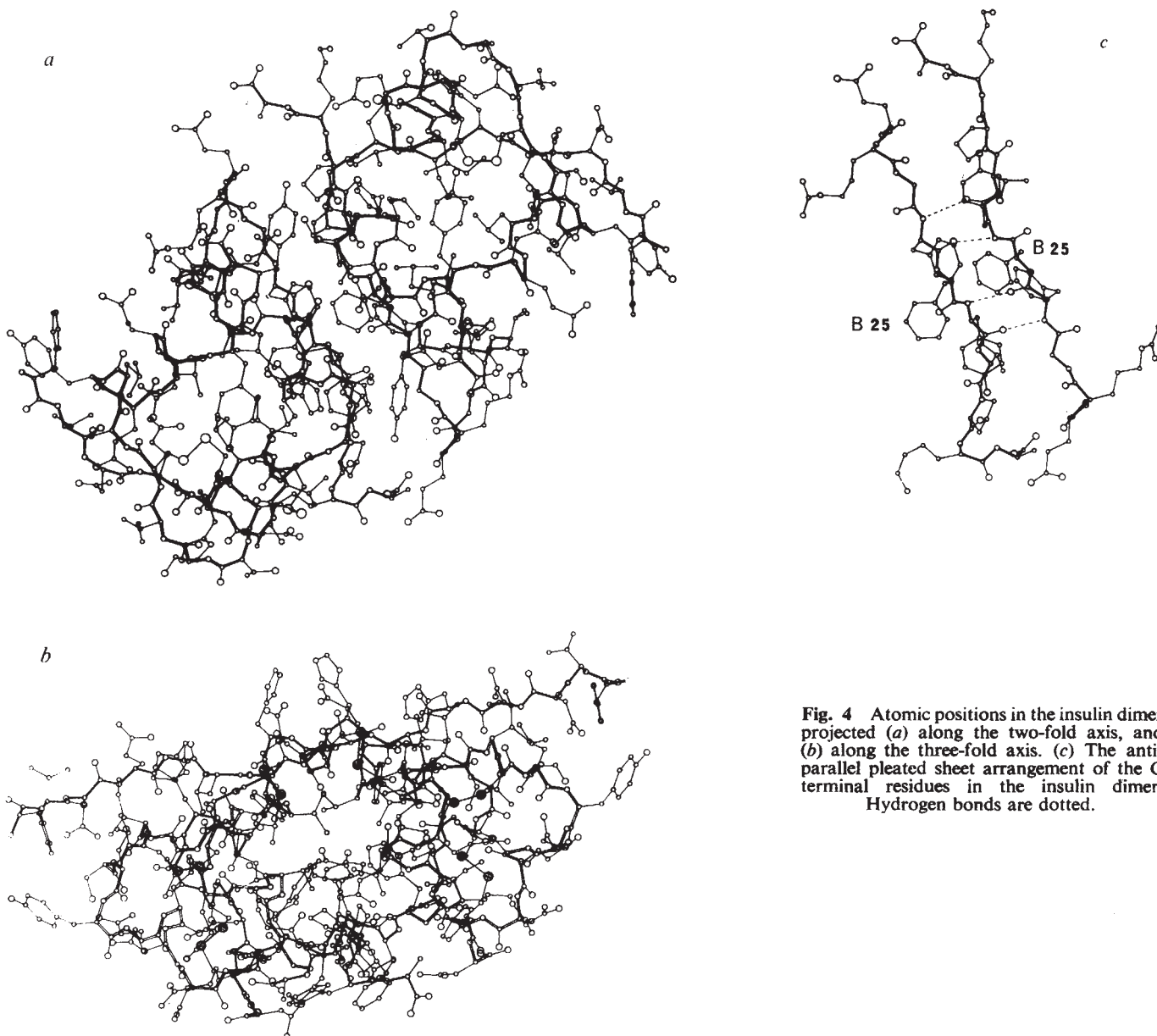


Fig. 4 Atomic positions in the insulin dimer projected (a) along the two-fold axis, and (b) along the three-fold axis. (c) The anti-parallel pleated sheet arrangement of the C terminal residues in the insulin dimer. Hydrogen bonds are dotted.

of the chains within the monomer. A second group, B 12 valine, B 16 tyrosine and B 24 phenylalanine (reinforced in most creatures by B 26 tyrosine and B 25 phenylalanine), is important in the contact between the two monomers formed on dimerization; they lie close to the dimer local two-fold

axis. A third group, A 1 glycine, A 5 glutamine, A 19 tyrosine and A 21 asparagine, are on the surface of the molecule in all the different states of aggregation of insulin. These are involved in interactions which may be important in maintaining the tertiary structure of the molecule, but they may

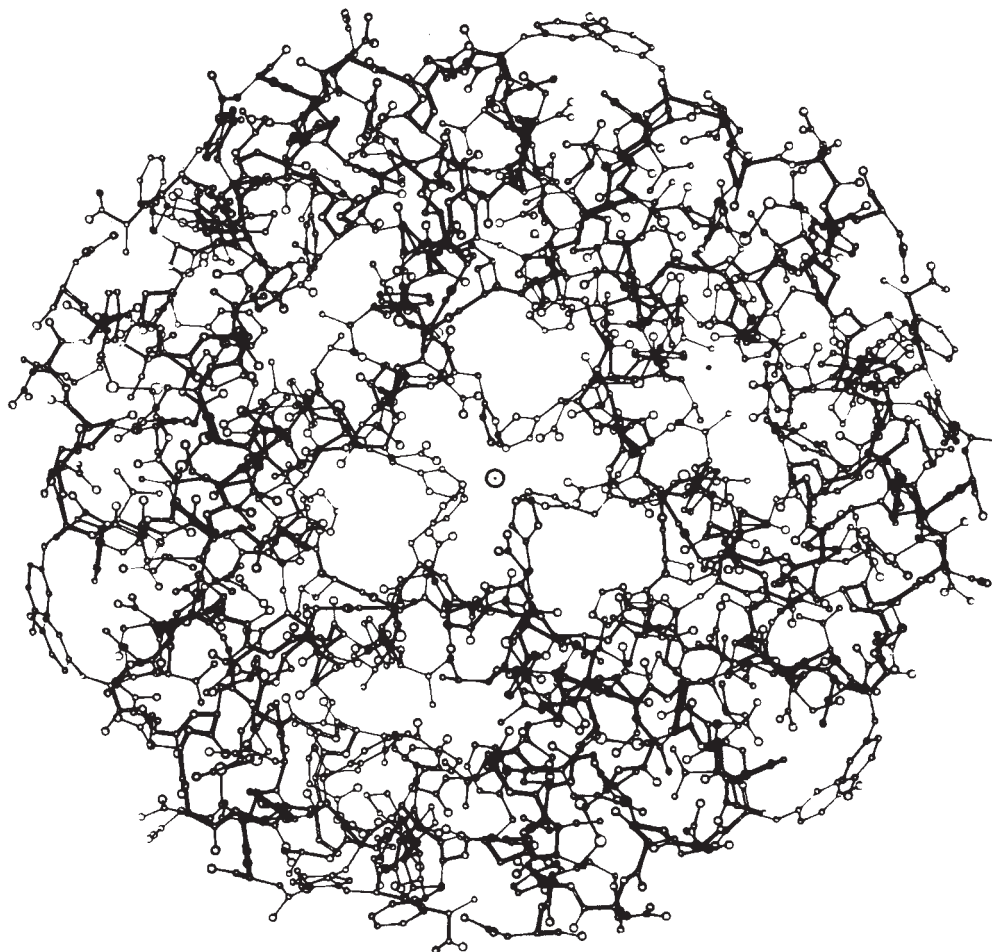


Fig. 5 The atomic positions in the insulin hexamer, projected along the three-fold axis.

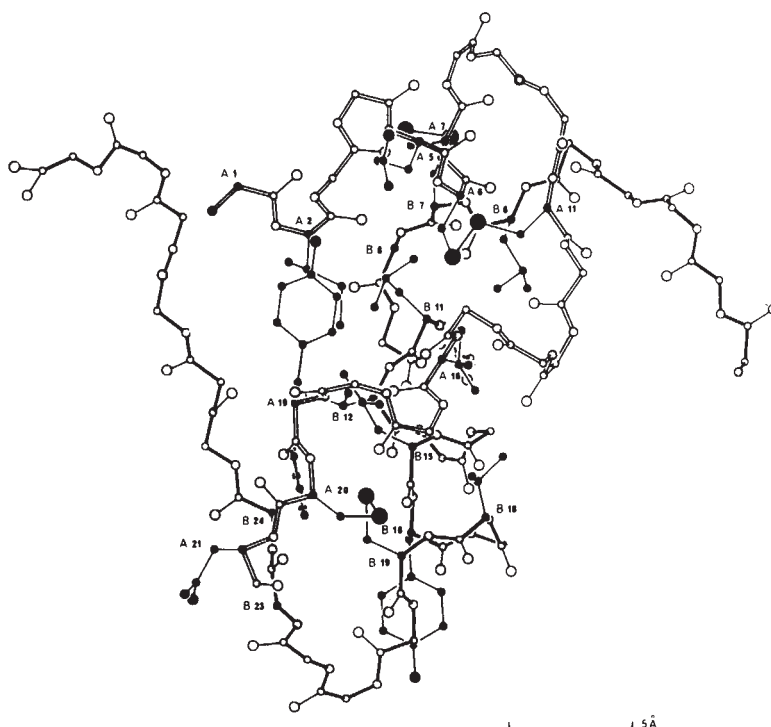


Fig. 6 The distribution of invariant residues in the insulin molecule, molecule 2, viewed perpendicular to the three-fold axis.

also be specifically involved in its activity; the removal of either the amino-group of glycine A 1 (Brandenburg, private communication) or the terminal asparagine, A 21 substantially inactivates the molecule⁸.

Many interesting relationships can also be observed in the residues which change in different insulins. These are usually on the surface and affect both the immunological properties of the insulin and the individual intermolecular contacts. Perhaps the most notable of the changes is the loss of histidine B 10 in guinea-pig and coypu insulins, which seems to be related to the absence of zinc in the islet cells⁹, and also perhaps to changes in B 14, B 17 and B 20, all residues in the dimer-dimer contact within the hexamers. It is possible that hexamers are not formed by these insulins, which again might suggest that hexamers are not necessary to biological activity—except that guinea-pig insulin is relatively inactive in the usual biological tests.

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Chromosomal Control of Reversion in Transformed Cells

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Malignant cell transformation and its reversion are controlled by the balance between two types of chromosomes that determine the expression of malignancy or its suppression. The chromosome groups that contain these types of chromosomes have been identified.

A MAJOR problem in understanding the mechanism of malignant cell transformation is to determine the genetic basis of the difference between normal and transformed cells, which results in the ability of transformed cells to form tumours *in vivo* and to multiply *in vitro* when the multiplication of normal cells is inhibited. Once these transformed properties have been hereditarily expressed in cells transformed by either viral or non-viral carcinogens, they can again be suppressed¹⁻³.

We have suggested³ that the expression or suppression of transformed properties depends on the balance between chromosomes carrying factors responsible for expression (E) and their suppressors (S). We have set out to test this model, and to identify the chromosomes carrying E and S, by the use of revertants from transformed cells with different degrees of suppression of transformed properties. The results provide

direct evidence for our model and indicate the chromosome groups that carry E and S.

Normal, Variant and Transformed Cells

Eight variants with a reversion of properties of transformed cells were examined together with normal and transformed cells for their properties *in vitro* (Table 1) and *in vivo* (Table 2). Reversion in these variants, as in those previously described^{1,2,4}, was not associated with a loss of the virus genome. The cells were tested *in vivo* for their ability to form tumours after subcutaneous inoculation into adult animals, and *in vitro* for their saturation density, cloning efficiency in liquid medium and soft agar, and the percentage of colonies at 41° C⁵ (41.1 ± 0.1° C³). The eight variants can be divided into three groups. The first group includes variants 1 and 2 which showed the highest degree of suppression of transformed properties. These variants produced 100% tumours only after inoculation of 10⁶ cells compared with 100% tumours after inoculation of 10³ transformed cells, and their saturation density, cloning efficiency in soft agar, and percentage colonies at 41° C, were the same as those found with normal cells. As will be shown later, the tumours produced by a variant such as variant 1 were formed by segregant cells with a chromosome constitution that is different from that characteristic for this variant.

The second group includes variants 3 and 4, which showed a somewhat higher but still low degree of expression of trans-

formed properties. The third group includes variants 5–8 which showed the highest degree of expression in the variants tested, although they still had a lower degree of expression than transformed cells (Tables 1 and 2).

Table 1 Saturation Density, Cloning Efficiency in Soft Agar and Liquid Medium, and Percentage Colonies at 41° C

Cell type	Time in culture (weeks)	Saturation density ($\times 10^{-5}$)	Cloning efficiency (%) in soft agar	on feeder layers at 37° C	% colonies at 41° C*
Normal	1	21	0†	2.5	0†
Variant 1	2	24	0†	24	0†
Variant 2	2	28	0†	26	0†
Variant 3	6	37	1	39	0.1
Variant 4	6	36	1	42	0.2
Variant 5	42	50	12	67	39
Variant 6	42	49	10	70	35
Variant 7	42	53	13	68	34
Variant 8	42	52	14	62	36
Transformed	> 50	145	34	93	76

Variants 1–8 were obtained from two clones from the hamster embryo cells hereditarily transformed by polyoma virus used in previous studies^{1–3}. Variants 5–8 were isolated by seeding cells from one clone of transformed cells on glutaraldehyde fixed normal cells⁴, and variants 1–4 were isolated from the other transformed clone after induction of variant formation at low cell density³.

* No. of colonies at 41° C

No. of colonies at 37° C $\times 100$.

† No. of colonies per 6,000 cells seeded.

had been in culture, with the highest degree of expression in variants which had been longest in culture (Table 1). The eight variants and transformed cells all had a similar growth rate in non-confluent cultures.

Total Chromosome Numbers

We have shown that reversion in six cases studied was associated with an increase in chromosome number³, and this has now been confirmed in two other revertants⁷. We have studied the chromosomes of twenty-eight revertants from polyoma virus-transformed cells, and have found that reversion can be associated either with an increase in chromosome number from diploid to subtetraploid or subhexaploid, or with a decrease in chromosome number to subdiploid. The eight variants used in the present study were all subdiploid.

Whereas normal cells had the expected diploid chromosome number of 44 (refs. 8 and 9), and transformed cells had modal numbers of 44 and 45, the modal chromosome numbers of variants 1–8 were 40 and 41, 42 and 43. The variants can be divided according to their chromosome number into three groups which are identical to those made according to the degree of expression of transformed properties. Variants 1 and 2 with the lowest degree of expression had modal numbers of 40 and 41, variants 3 and 4, which showed a higher degree of expression, had a higher modal number of 42, and variants 5–8 with the highest degree of expression, had the highest modal number of 43 (Table 3).

Chromosome Groups that Carry E and S

We have studied the karyotypes of the variants, and of normal and transformed cells. Chromosomes were divided into

Table 2 Tumour Formation in Adult Animals

Cell type	10 ³		No. of cells inoculated per animal *				10 ⁶	
	Tumour incidence	Average latency period (days)	Tumour incidence	Average latency period (days)	Tumour incidence	Average latency period (days)	Tumour incidence	Average latency period (days)
Normal	0/5†	—	0/5	—	0/5	—	0/5	—
Variant 1	0/4	—	0/4	—	2/5	36	5/5	30
Variant 2	0/4	—	0/4	—	1/5	36	5/5	31
Variant 3	0/4	—	2/4	36	4/5	25	5/5	14
Variant 4	0/4	—	2/4	35	5/5	23	5/5	14
Variant 5	1/4	44	4/5	23	5/5	17	5/5	11
Variant 6	2/4	46	5/5	21	5/5	17	5/5	11
Variant 7	1/4	45	4/5	21	5/5	17	5/5	11
Variant 8	1/4	46	4/5	23	5/5	17	5/5	10
Transformed	5/5	34	5/5	18	5/5	10	5/5	7

* The cells were inoculated subcutaneously into 4–5 week old hamsters in 0.2 ml. of Eagle's medium. The animals were tested for progressively growing tumours twice weekly for 50 days. The latency period for tumour development was taken as the time when the tumours first become palpable.

† No. of animals with tumours/No. of animals inoculated.

The three groups of variants showed, in relation to their degree of expression, differences in the degree of agglutination by concanavalin A. This carbohydrate-binding protein agglutinates transformed cells but not normal cells in the same conditions⁶. Variants 1 and 2, like normal cells, showed no agglutination by this agglutinin.

All the variants 1–8 had an epithelioid morphology¹, in contrast to the fibroblastic morphology of the normal and transformed cells. The degree of expression of transformed properties in these variants was related to the time that the cells

fourteen groups according to their size and location of the centromere (Fig. 1). Normal cells contain chromosome groups 1–8, so that groups 9–14 consist of unusual chromosomes produced by chromosome re-arrangement. The karyotypes have shown (Table 3) that the number of chromosomes in groups 5 and 9 was related to the degree of expression.

A decreased degree of expression was correlated with a decrease in the number of chromosomes in group 5 (Table 3, Fig. 2). This decrease in expression was also correlated with a decrease in the proportion of group 5 chromosomes in the cells,

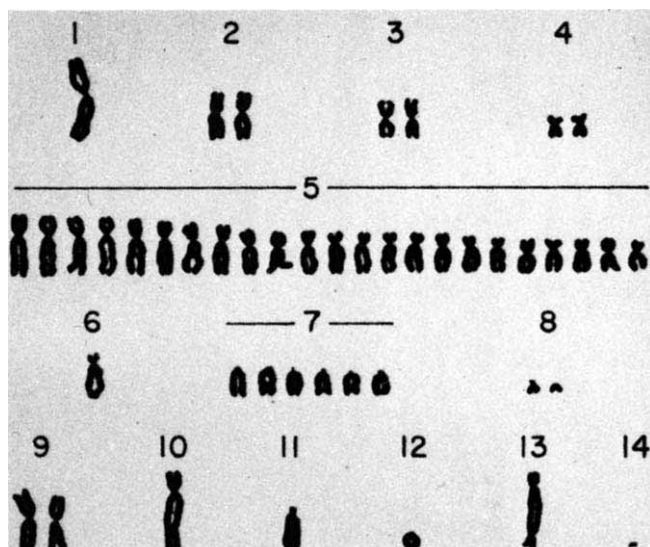


Fig. 1 Grouping of chromosomes according to their size and position of the centromere. Groups 1-10 in this karyotype are from one cell of variant 3. Groups 11-14 are from other cells. Normal cells contain groups 1-8. ($\times 1,750$.)

which indicates that the decrease in group 5 was not caused by a random chromosome loss. The results further show that the transformed cells tested had a higher number (Fig. 2) and proportion of group 5 chromosomes than normal cells. The same pattern was found in a line of hamster cells transformed after treatment with the chemical carcinogen dimethylnitrosamine. These chemically transformed cells again showed an increase in the number of group 5 chromosomes, and reversion² was associated with their loss. These results therefore indicate that chromosomes in group 5 carry the factors for expression E.

A decreased degree of expression in the variants was also associated with an increase in the number of chromosomes in

group 9 (Table 3, Fig. 2). Because chromosomes from this group were not found in the normal or transformed cells, the group 9 chromosomes in the variants must have been produced by re-arrangement of chromosomes in one or more other groups. In the variants used for the data in Table 3 and also in subtetraploid variants, the appearance of group 9 chromosomes was always associated with a decrease in the number of chromosomes in groups 6 and 7. This indicates that group 9 originated from groups 6 and 7. Data obtained with subdiploid variants from cells transformed after treatment with dimethylnitrosamine support this assumption, because in these variants there were no group 9 chromosomes and there was also no decrease in the number of chromosomes in groups 6 and 7. These results, therefore, indicate that the factors for suppression S are carried on groups 6 and 7 in normal and transformed cells, and also on group 9 in the variants from polyoma transformed cells.

The cells of variant 1 showed a complete suppression of the *in vitro* transformed properties shown in Table 1, and also showed, like normal cells, no agglutination by 500 $\mu\text{g}/\text{ml}$. of concanavalin A⁶. But some tumours were obtained in animals after inoculation of 10^5 cells (Table 2). We have studied the karyotypes of three tumours derived from variant 1. In contrast to the modal chromosome number of 40 for variant 1, the cells of tumour 1, which were examined 7 weeks after inoculation, had a modal number of 42, and tumours 2 and 3, which were examined 14 weeks after inoculation, had 14 and 20% of cells with 42 chromosomes, and modal numbers of 78-82 and 76-85, respectively. A comparison of the karyotypes of the tumours and variant 1 has shown (Table 4) that there was in the tumours an increase in the number of chromosomes in group 5 and a decrease in the number of chromosomes in group 9, both in the subdiploid and in the subtetraploid cells. The cells from these tumours showed a high tumorigenicity, and produced 100% tumours after inoculation of 10^3 cells.

These data on the tumours again indicate the location of E and S on groups 5 and 9, respectively. They also show that tumours, formed after inoculation of a variant with complete

Table 3 Number of Chromosomes in Groups 1-14 in Normal, Variant and Transformed Cells

Cell type	Modal chromosome number	Group Nos.*										
		1	2	3	4	5	6	7	8	9	10	11
Normal male	44	1	2	2	2	25	2	8	2	0	0	0
Normal female	44	2	2	2	2	24	2	8	2	0	0	0
Variant 1	40	2	2	2	2	21	1	5	2	3	0	0
Variant 2	40, 41	1	2	2	2	21, 22	0	6	2	3	1	0
Variant 3	42	1	2	2	2	22, 23	1	6	2	2	1	0
Variant 4	42	1	2	2	2	22-24	1	6	2	2	1	0
Variant 5	43	1	2	2	2	25	1	6	2	1	1	0
Variant 6	43	1	2	2	2	25	1	6	2	1	1	0
Variant 7	43	1	2	2	2	25	1	6	2	1	1	0
Variant 8	43	1	2	2	2	25	1	6	2	1	1	0
Transformed	44, 45	0	2	2	2	26	2	8	2	0	0	1

For the chromosome analysis, 4 $\mu\text{g}/\text{ml}$. colchicine was added to exponentially growing cells for 2 h. After trypsinization, the cells were centrifuged, suspended in a 1% sodium citrate solution for 20 min at 37° C, fixed in a solution containing 1 part glacial acetic acid and 3 parts absolute ethyl alcohol, air dried, and stained with Giemsa. Fifty cells were counted for the chromosome numbers and twenty for the karyotypes.

* The number of chromosomes in each group are the modal numbers. When there was one modal number, this number was found in 50-100% of the cells. When there was more than one modal number, the numbers given were found in at least 70% of the cells. There was one chromosome of group 12 in 4-12% of the cells in variants 3, 5, 6, 7, 8, and transformed cells; one chromosome of group 13 in 4-15% of the cells in variants 1, 2, 3, 5, 6, 7, and transformed cells, and 6% cells with 2 such chromosomes in variants 7 and 8; and there was one chromosome of group 14 in 56 and 6% of the cells in variants 2 and 6, respectively.

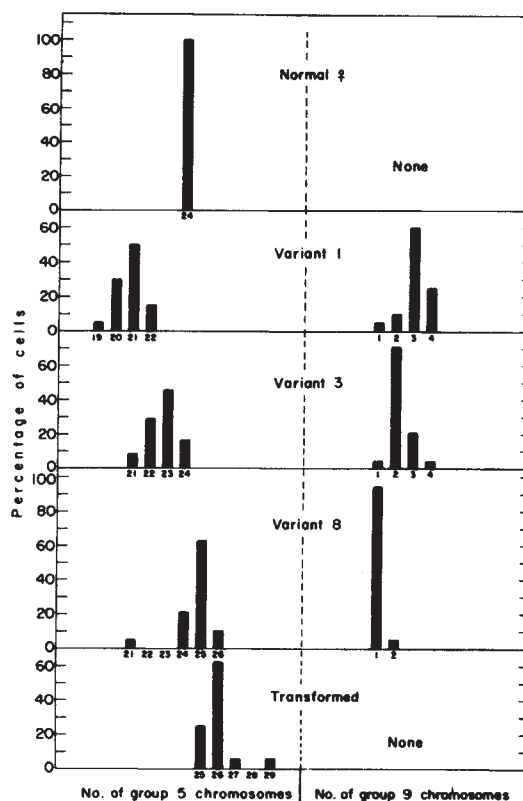


Fig. 2 Number of chromosomes in groups 5 and 9 in normal, variant and transformed cells.

suppression of *in vitro* transformed properties, were produced by segregants with a chromosome constitution different to that of the variant. It can thus be concluded that tumorigenicity in this variant was also completely suppressed.

Although tumours were produced in animals after inoculation of many cells of variant 1, there were no tumours after inoculation of the same number of normal cells (Table 2). This indicates that the chromosome constitution of the suppressed variant was less stable than that of the normal cells, and was more readily able to undergo changes in chromosome balance that resulted in an excess of E over S.

E and S. Whether diploid normal cells contain an equal amount of E and S or an excess of S can be determined by cell fusion. The suppression of transformed properties in hybrids produced by cell fusion of diploid normal cells and transformed cells, without further chromosome change in the hybrid, would indicate that the normal cells have an excess of S.

In addition to these studies, exponentially growing cells were treated with 3 µg/ml. of 5-bromodeoxyuridine for 48 h followed by illumination with visible light for 80 min¹³. After this treatment, the percentage survivors of variants 1, 3 and 8, and transformed cells, were 81, 70, 18 and 0, respectively. These

Table 4 Number of Chromosomes in Groups 1-14 in Tumours derived from Variant 1

Cell type*	Chromosome No.	Frequency in population (%)	1	2	3	4	Group Nos. †		7	8	9	10
							5	6				
Subdiploid cells												
Variant 1	40	58	2	2	2	2	21	1	5	2	3	0
Tumour 1	42	52	1	2	2	2	22, 23	1	6	1	2	1
Tumour 2	42	14	1	2	2	2	23	1	6	2	2	1
Tumour 3	42	20	1	2	2	2	23	1	6	2	2	1
Subtetraploid cells												
Variant 1	75-79	20	2	4	4	4	40, 42, 43	2	10	2	6	0
Tumour 1	82-86	6	2	4	4	4	44-48	2	12	2	3, 4	2
Tumour 2	78-82	70	2	4	3	2, 3	45-47	2	10-12	4	3, 4	1-3
Tumour 3	76-85	74	2	4	3, 4	4	43-46	2	12	4	4	1-3

* The tumours were cultured for 3-4 days after removal from the animals.

† There was one chromosome of group 11 in 5% of the subtetraploid cells in tumour 3; one chromosome of group 12 in 100 and 17% of the subdiploid cells in tumours 1 and 3, and in 17-50% of the subtetraploid cells in tumours 1, 2 and 3, and two chromosomes of group 12 in 5-50% of the subtetraploid cells in tumours 1, 2 and 3; and there was one chromosome of group 14 in 10 and 5% of the subtetraploid cells in tumours 2 and 3, respectively.

Mechanism of Transformation and Reversion

Our data provide direct evidence for the model that the expression and suppression of transformed properties depend on the balance between chromosomes with E and S. Transformation in the polyoma transformed cells was associated with an increase in the number of chromosomes with E which established an excess of E over S. The delay in expression of transformed properties following the treatment of normal cells with carcinogen¹⁰ may correspond to the time required for the preferential increase in the chromosomes with E.

Reversion in the subdiploid variants was associated with a specific chromosome loss which resulted in an excess of S over E. This excess was sufficient to suppress the transformed properties, in spite of maintenance of the viral genome in reverted cells^{1,2}. The treatment of cells with agents which change the chromosome balance so as to produce an excess of S may thus be of value in tumour therapy. During cultivation *in vitro*, variants underwent re-reversion. This was caused by a specific increase in chromosome number which resulted in a progressive increase of E over S. There was a similar increase in tumours produced by re-revertant segregants from a variant with a complete suppression of the ability for tumour formation. It is still to be determined whether every chromosome in group 5 carries E, and every chromosome in groups 6, 7 and 9 carries S, and whether there can also be non-chromosomal E and S.

Chromosome combinations that lead to the suppression of transformed properties have also been obtained by cell fusion between the subtetraploid mouse cell lines 3T3 (ref. 11) or A9 (ref. 12) and mouse transformed cells. Re-reversion in the progeny of fused cells was associated with chromosome loss¹², which presumably produced a change in the balance between

results indicate that the balance between these chromosomes with E and S may also control the regulation of other cellular properties.

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LETTERS TO NATURE

PHYSICAL SCIENCES

Two Bright New Quasi-stellar Radio Sources

A 2,695 MHz survey of radio sources in a narrow strip of declination has recently been completed with the Jodrell Bank Mk II radio telescope (full details will be published later). Accurate positions for all the 74 sources have been measured with the RRE Malvern 2,695 MHz interferometer. Using these positions and a transparent overlay technique of identification the relative positions of radio and optical objects were located on the National Geographic-Palomar Sky Survey prints with an r.m.s. error of 4 arc s. In the 24 h of right ascension covered by the survey, twenty-three identifications with stellar objects have been made; among these are two remarkably bright (14.5 magnitude) objects, separated by less than two degrees in the sky, which may be worthy of special attention. Finding charts for these two objects are shown in Fig. 1.

The radio position of the two sources, B2 1215+30 (ref. 1) and ON231 (ref. 2), together with the displacements of the optical objects from the radio positions are given in Table 1. Each proposed identification lies within one standard error (4 arc s) of the radio position. Using the densities of stars quoted by Dewhurst³ the probability of finding a normal star brighter than 14.5 magnitude within 10 arc s of the radio position is estimated to be approximately 0.002. The magnitudes of the objects were estimated from the red Sky Survey prints. Comparison of the red and blue print images of the

objects indicates that they are both of neutral colour. There is faint nebulosity lying to the south of B2 1215+30 (Fig. 2a) and there is a possible indication that the main image is slightly non-stellar on the red print.

Table 1 Radio and Optical Positions of B2 1215+30 and ON231

Source	Radio position		Optical position minus radio position arc s	
	RA 1950	December 1950	RA	December
B2 1215+30	12 15 21.3±0.15	30 23 39±2.0	0	+3
ON231 *	12 19 01.06±0.06	28 30 35.9±0.6	+4	-4

* Position measured by Adgie, Crowther and Gent (private communication).

ON231 is completely stellar on both the red and the blue Sky Survey prints, but there is an 18 magnitude galaxy-like object which might be described as a "jet" lying 12 arc s from the main star (Fig. 2b). It has been pointed out by Dr B. Pagel (private communication) that the optical object identified with ON231 is W Coma Berenices. This variable was found by Wolf⁴ and reported to range in brightness from 11.5 to 14 optical magnitude. It is also listed in the *General Catalogue of Variable Stars*⁵ where it is quoted as varying between 13 and 15.5 magnitude. No other published information on the optical properties of this object appears to be available.

The little radio spectral information available for the two sources is summarized in Table 2. The 2,695 MHz fluxes were measured with the Jodrell Bank Mk II telescope. 1,415

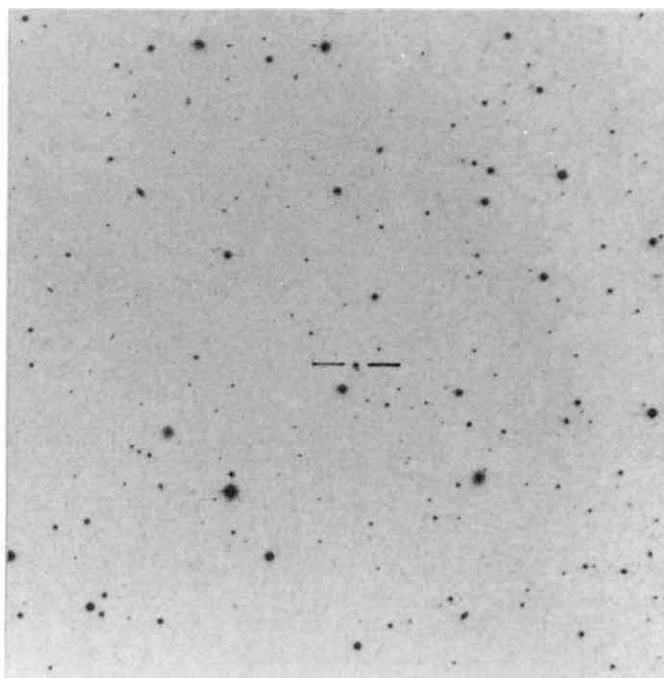
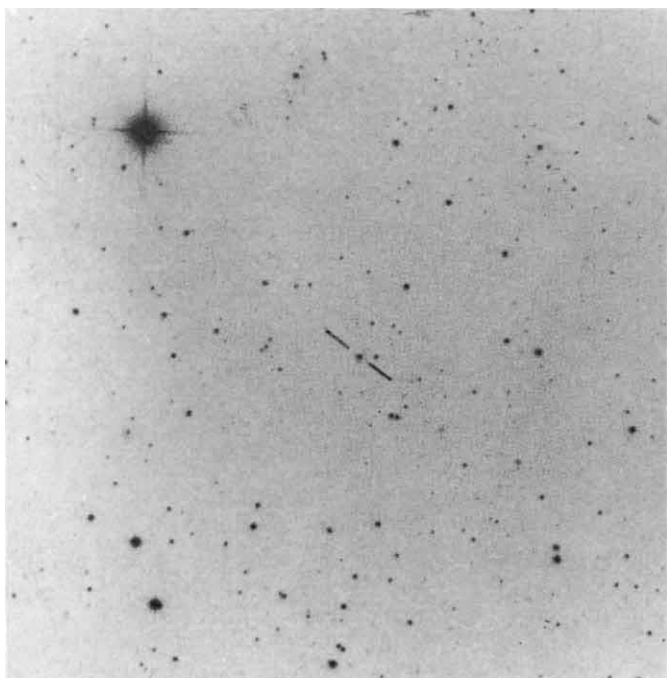


Fig. 1 Finding charts, reproduced from the red print of the sky survey (copyright National Geographic Society-Palomar Observatory), for B2 1215+30 (left) and ON231 (right).

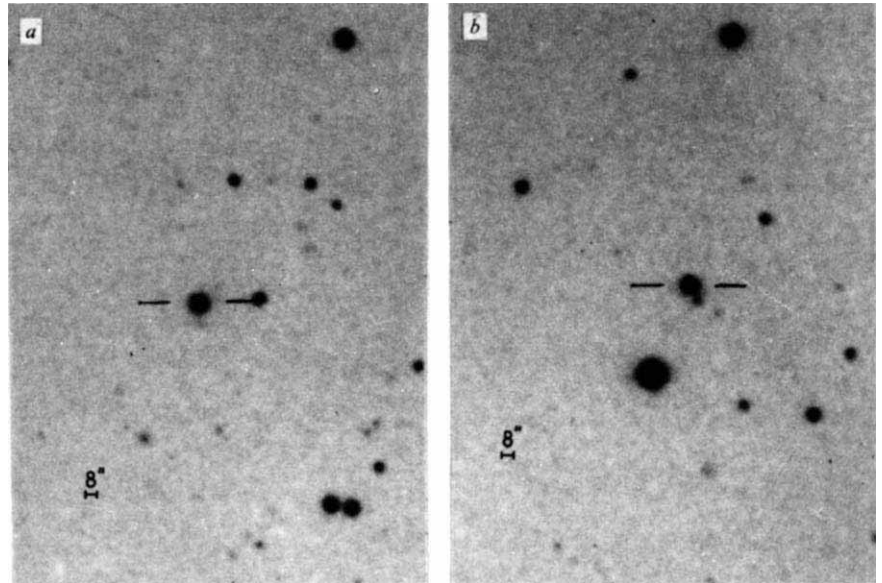


Fig. 2 Enlargements of the fields around the two sources: *a*, B2 1215+30; and *b*, ON231. The error bars show the combined r.m.s. uncertainty of the optical and radio measurements (± 4 arc s).

MHz fluxes are taken from Scheer and Kraus² and the 408 MHz flux of B2 1215+30 is taken from the Bologna survey¹. From the limited data available it is possible to say with reasonable certainty that ON231 has an inverted spectrum, while that of B2 1215+30 is relatively flat. Both sources were unresolved when observed with a 1,000 wavelength interferometer.

Examination of the literature reveals that there are only four other radio sources which have reasonably certain associations with stellar objects of 14.5 magnitude or brighter. These objects are listed in Table 3 with some of their important properties and references to finding charts. Similarities exist between these four sources and the two new objects: all have radio spectra which are either flat or inverted and three of the objects, including the two new ones, are of neutral colour. Furthermore, VRO 42.22.01 is identified with the "star" BL Lacertae which is known to vary irregularly between 12 and 15.5 magnitude. Thus, the resemblance between ON231 and VRO 42.22.01 is particularly striking. Additional conclusions as to the true nature of ON231 and B2 1215+30 must await studies of the optical spectra of these objects and research into the variability of their optical and radio emission.

Finally, if the source ON231 is a quasar and the object in close proximity to it turns out to be a field galaxy, then neutral hydrogen absorption due to the galaxy should be detectable in the radio spectrum of the source, provided it is located beyond the galaxy. If this absorption were detected it would lend support to the cosmological interpretation of quasar redshifts.

Table 2 Flux Densities and Spectral Indices (defined as α where $S \propto \nu^\alpha$ and S is the Flux Density at Frequency ν) of B2 1215+30 and ON231

Source	Flux density in units of 10^{-26} W m^{-2} Hz $^{-1}$			Spectral index
	408 MHz	1,415 MHz	2,695 MHz	
B2 1215+30	0.84 ± 0.05	0.46 ± 0.13	0.47 ± 0.07	-0.3
ON231	—	0.44 ± 0.13	0.80 ± 0.07	+0.9

Table 3 Properties of Other Sources identified with Bright Stellar Objects

Source	Magnitude	Colour	Radio spectrum	Reference
3C 273	12.8	Blue	Inverted	6
VRO 42.22.01	12-15.5	Slightly blue	Inverted	7
OJ 287	14.5	Neutral	Inverted	8
4C 31.63	14.5	Blue	Flat	9

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Isotropy of the 3 K Background

THE 3 K background radiation provides a frame of reference equivalent to that defined by the matter by which it was last scattered. Conservative estimates place this last scattering at a redshift of $z=7$ while others range up to redshifts of 1,000 or so^{1,2}. Motion of the Earth relative to this extremely distant reference frame can be measured by observing the 24 h anisotropy in the 3 K background. If we assume no intrinsic anisotropy in the radiation, then a velocity of the Earth v would result in a temperature distribution

$$T(\theta) = T_0 \left(1 + \frac{v}{c} \cos \theta \right) \quad (1)$$

where T_0 is the average background temperature and θ is the angle between v and the direction of observation³. Thus a temperature anisotropy of 10^{-3} K (1 mK) corresponds to a speed of ~ 100 km s $^{-1}$. Previous efforts to measure this anisotropy have yielded only the component in the Earth's equatorial plane^{4,5}. The most precise result to date is that obtained by Conklin, who reports an equatorial component of 1.9 ± 0.8 mK directed toward 10 h right ascension. (This

result (unpublished) is based in part on data obtained after publication of ref. 5.) The experiment described here is an attempt to determine the right ascension, declination and magnitude of the Earth's velocity vector by measuring the 24 h anisotropy in the cosmic background.

Measurements were made with an X-band Dicke radiometer⁶ carried by balloon to 24 km altitude to minimize noise due to atmospheric emissions. The receiver was a tunnel diode amplifier-detector chain with a noise figure of 5 dB and a pass band from 9.9 to 10.4 GHz. This frequency range was selected because it is a relatively unused portion of the X-band. Interference due to out-of-band emissions was eliminated by a bandpass filter, which provided 75 dB attenuation below 9.6 and above 10.7 GHz. The input to the receiver was switched at 2 kHz between two broad-beam (15° FWHM) horn antennas tipped in opposite directions at 45° from the zenith. The entire radiometer rotated about a vertical axis at 1 r.p.m. Its orientation was monitored from the telemetered outputs of two flux-gate magnetometers affixed to the gondola. Thermal emission from the Earth was attenuated by aluminium shielding screens beneath the antennas. To avoid the large solar and lunar microwave power fluxes, the radiometer was flown on a moonless night. The twelve hours at altitude allowed observation of about half the northern celestial hemisphere, from ~3 h to ~15 h right ascension.

The basic scheme of the data analysis was to look for a periodicity in the telemetered radiometer output synchronous with the gondola rotation, and then find the parameters of a 24 h anisotropy which fit this periodicity best. For every rotation of the gondola the radiometer output was Fourier-analysed into its fundamental frequency sine and cosine components, using magnetic north to define the zero of angle. Thus the cosine coefficients represented the north-south antenna temperature differences, while the sine coefficients were a measure of the east-west differences. The Earth's magnetic field coupled with the antenna switch causing a spurious sinusoidal output of the radiometer as the gondola rotated. Although the amplitude of this effect, typically 15–20 mK, could not be calculated precisely enough to permit reliable subtraction of it from the data, its phase was accurately known. Ground tests showed that the perturbation depended primarily on the component of the magnetic field normal to the symmetry plane of the two antennas. The measured sensitivity of the switch to parallel field components was less than 2% of its sensitivity to the perpendicular component. Thus the phase of the perturbation was such that it affected the sine coefficients, but was 90 degrees out of phase with the cosine components and, hence, made no contribution to them. The cosine terms, then, constituted a "clean" data set which could be further processed for evidence of a background anisotropy.

The effect of galactic radiation was estimated by assuming a (temperature) spectral index of -2.80 and scaling a 404 MHz map of the galaxy⁷ to 10.15 GHz. Computer-simulated radiometer scans, corrected for the difference between magnetic and true north, were made on this map and the resultant cosine coefficients subtracted from the data. The residuals, averaged over hourly intervals, are shown in Fig. 1.

From the data displayed in Fig. 1 an estimate of the background anisotropy can be made. One sees that the data do not support the hypothesis of an isotropic background. The fit of the data to a null result yields a χ^2 of 33. A crude idea of the magnitude of the anisotropy is obtained by taking the average of all the data. The fit to such a horizontal straight line yields a mean value of -3.3 ± 1.2 mK with a χ^2 of 20. To state the result differently, when an antenna looked south it saw, on the average, a sky temperature 3.3 mK hotter than that seen by the other antenna looking north. To obtain more detailed information about the anisotropy requires the fitting of the data to a three-parameter model of a 24 h anisotropy. Using the method of maximum likelihood⁸ we find the best-fit parameters and their associated single standard deviation

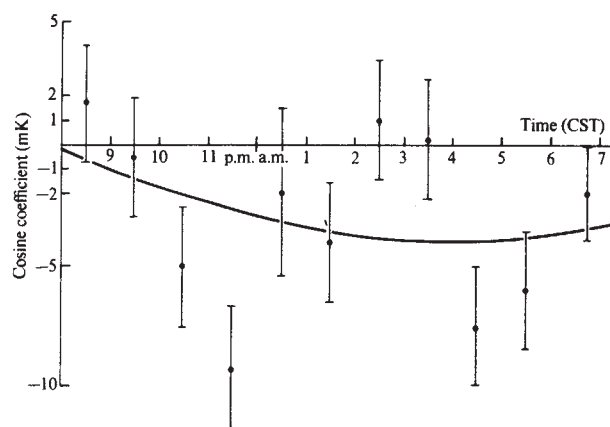


Fig. 1 Hourly averages of cosine coefficients corrected for galactic radiation. The curve is the best fit to a 24 h anisotropy. Error bars represent single standard deviation error limits.

errors as follows: $10\frac{1}{2} \pm 4$ h right ascension, $-30 \pm 25^\circ$ declination, and 3.2 ± 0.8 mK amplitude. The curve corresponding to these parameters is shown in Fig. 1. The χ^2 for this fit is 18. The single standard deviation errors quoted above are the usual 68% probability limits based on the random fluctuations in the data.

Table 1 Anisotropy Parameters for Different Spectral Indices

Spectral index	Right ascension (h)	Declination ($^\circ$)	Amplitude (mK)
-2.65	10	-40	2.8
-2.80	$10\frac{1}{2}$	-30	3.2
-2.95	$11\frac{1}{2}$	-30	3.0
$-2.7 - 0.2 \sin^2 b$	11	-30	3.3

The most likely source of systematic error is uncertainty in the galactic spectral index. It was found, however, that over a wide range of galactic models the best-fit anisotropy parameters remained substantially unchanged, as is shown in Table 1. The first three entries span a range of -2.65 to -2.95 for the spectral index. This interval includes most of the published estimates of the index⁹⁻¹¹. Because of HII concentrations in the plane of the galaxy, one might suggest that the index is dependent on galactic latitude. There is no firm evidence that this is the case, but to allow for such a possibility an index of $-2.7 - 0.2 \sin^2 b$, where b is the galactic latitude, was tried. The results are shown in the last entry of Table 1.

Although none of the spectral indices discussed so far can account for the observed anisotropy in sky temperature, it is possible to define, *ad hoc*, a model that will. Assume the index to be -2.80 everywhere except within a circle of radius 45° centred on $10\frac{1}{2}$ h, -30° , where it is -2.60 . The galactic "hot spot" resulting from this index would be indistinguishable in this experiment from a true cosmic anisotropy. Such spatial structure of the index has not been observed, but considering the uncertainties in its measurement to date, the possibility cannot be ruled out.

As well as the uncertainty in the spectral index, there are three possible sources of systematic error that deserve comment. (1) The calibration of the radiometer was monitored during the flight and found to be constant to within 10%. A calibration error of this magnitude would cause no appreciable shift in the calculated anisotropy parameters. (2) The freedom of the cosine coefficients from the magnetic perturbation was verified in a series of ground tests. These showed that the error due to any magnetic effects was less than the single standard deviation random error limits associated with the anisotropy parameters. (3) Possible man-made interference was investigated with the assistance of the Electromagnetic Compatibility

Analysis Center in Annapolis, Maryland. All transmitters, civilian and military, operating in the USA are registered with this organization. A check was made of any equipment that might have significantly perturbed the radiometer. Most of these devices were used only rarely, and it was verified that on the night of the flight no registered transmitter within the horizon of the radiometer was in operation.

The results of this experiment corroborate the findings of Conklin mentioned earlier. The observed anisotropy can also be compared with astronomical estimates of the Earth's motion, including the effects of the local supercluster¹²⁻¹⁴. These indicate a speed of about 400 km s^{-1} in the direction of $14 \text{ h}, -20^\circ$. The uncertainty in these figures is difficult to assess, but a reasonable estimate would be: $\pm 200 \text{ km s}^{-1}$, $\pm 2 \text{ h}, \pm 20^\circ$. The agreement of this velocity with the one corresponding to the anisotropy parameters stated earlier (320 km s^{-1} , $10\frac{1}{2} \text{ h}, -30^\circ$) supports Conklin's assertion that there is no large motion of the local supercluster relative to the more distant frame defined by the 3 K background radiation.

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Asymmetric Seafloor Spreading south of Australia

SINCE mid-1968, the National Science Foundation research vessel *Eltanin* has operated south of Australia on a systematic geological, geophysical, and oceanographic reconnaissance. North-south tracks at a spacing of 5° longitude or closer transect the area of interest from 140° E to 105° E . The *Eltanin* data are supplemented by geophysical data from Lamont-Doherty ships, from Australian, French and Japanese sources, and from aeromagnetic tracks.

An earlier account of seafloor spreading between Australia and Antarctica¹ was based on limited data, confined almost entirely to the north flank of the Indian-Antarctic ridge. A detailed study of the magnetic anomaly lineation pattern for this entire region is now in progress (J. K. W. *et al.*, to be published). The pre-drift reconstruction of Australia and Antarctica has been re-examined² by fitting the 2,000 m isobaths of the respective margins in the fashion of Bullard *et al.*³. There is no morphological or geological evidence for crustal

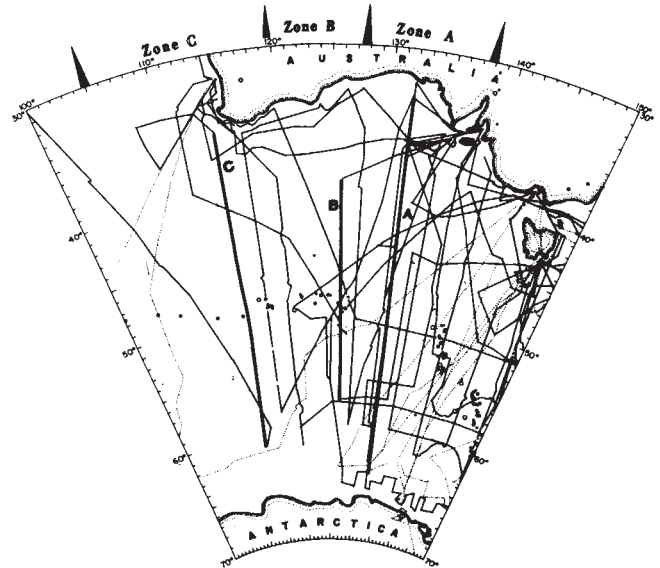


Fig. 1 Index map of tracks along which geophysical data have been obtained. Relocated earthquake epicenters⁵ are shown as circles, the smallest representing the most accurate determinations. Boundaries of the inferred zones of contrasting geophysical and morphological properties are indicated. Bold lines identify the profiles shown in Fig. 2. Solid tracks are those of Lamont ships or programmes (r.v. Vema, Robert D. Conrad, Eltanin). Dotted tracks are those from all other sources. Stereographic conformal projection.

subduction zones along the continental margin of Australia or along that of Antarctica; the very thick accumulations of sediment that exist beneath the Wilkes Abyssal Plain and seem to thicken toward the south may, however, be indicative of an ancient buried trench (R. Houtz, private communication). The seismic activity of this region has been reviewed⁴ and relocated epicenters⁵ are shown in Fig. 1.

In this article we will consider briefly the large amount of new data for this area. We recognize the presence of three longitudinal zones, distinctly different in their geophysical and morphological properties, which constitute integral parts of two of the principal crustal plates (Indian Plate and Antarctic Plate). Their contrasting properties suggest that the basic concepts of plate tectonics (that is, large, rigid lithospheric plates, undeformed except at their boundaries), though convincingly demonstrated on a global scale, may not strictly hold in a detailed analysis. The demonstration of systematic asymmetric spreading is the most important method to be dealt with in this article.

Zone A (138° E to 128° E) is almost aseismic, implying an absence of important transform faults. Aseismic zones of similar extent also characterize segments of the East Pacific Rise^{4,6}. Zone B (128° E to about 120° E), however, shows a broad zone of seismic activity. Zone C also exhibits seismic activity—to a smaller extent than zone B—and is confined to a narrower zone⁵. There is no linear grouping of epicenters in either zones B or C of the kind that might define large active transform faults. The presence of small offsets of the ridge crest is therefore inferred for both zones B and C.

Fig. 2A shows a typical profile of topography and total intensity magnetic anomalies for zone A from the Antarctic continental slope to the Australian continental slope. Several key anomaly lineations⁷ can be identified. The classic morphological expression of a mid-oceanic ridge with an easily recognizable crestal zone is typical of zone A. The local topographic relief here is relatively low at about 100 to 200 m although a few large peaks are present on the southern flank. The magnetic lineations in this zone are well defined and the pattern can be traced both north and south from the axial zone at least to anomaly 13 and probably to anomaly 21.

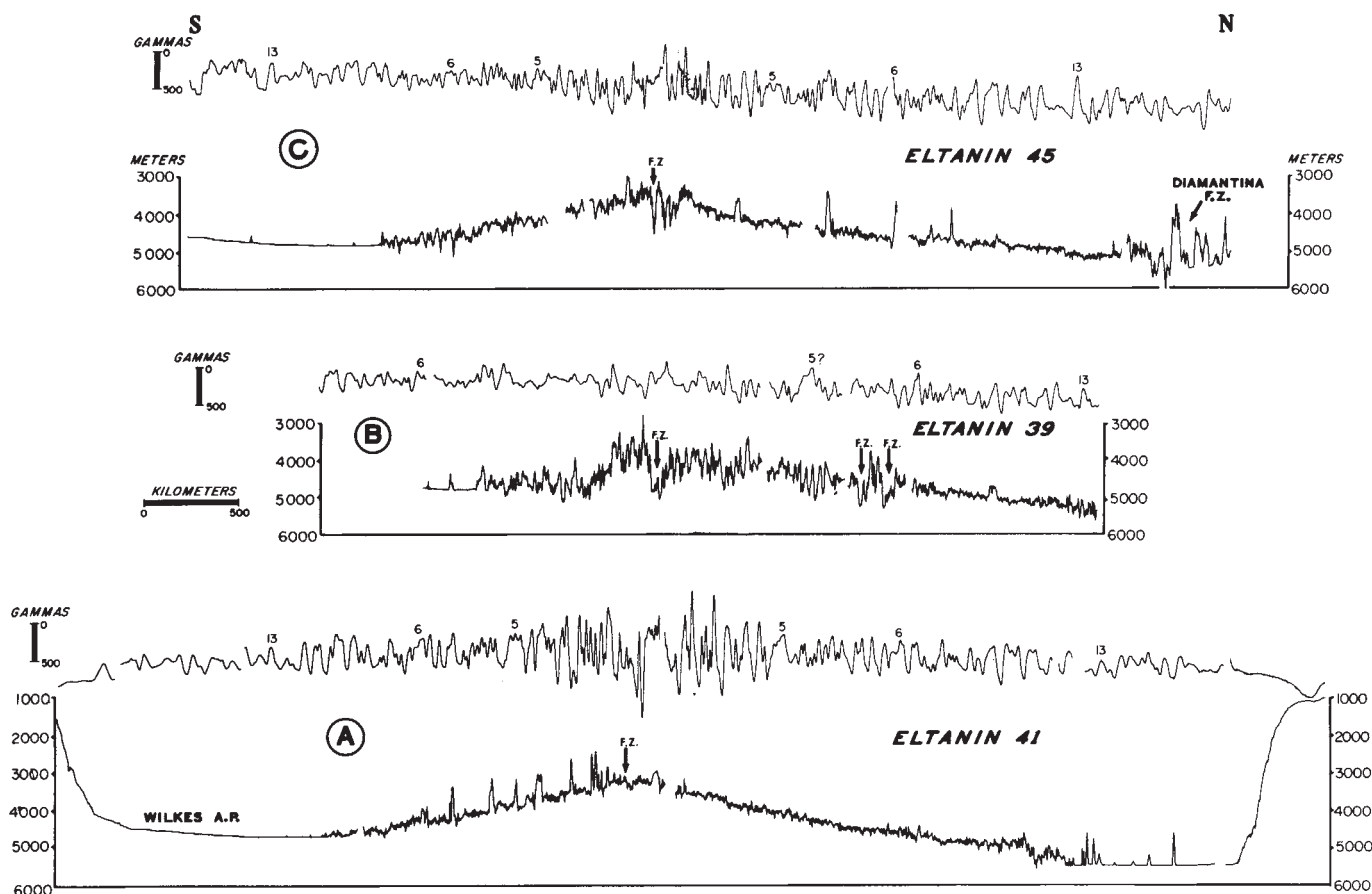


Fig. 2 Representative magnetic and topographic profiles from each of the three zones. These are projected along a constant azimuth of average spreading direction determined from centres of rotation. Physiographic expressions of probable fracture zones are denoted by FZ.

There are a number of "magnetic quiet zones" which begin just seaward of the continental slope and extend towards the land across the Antarctic and Australian continental margins.

Fig. 2B shows representative profiles from zone B. The area is characterized by very high amplitude topographic relief (about 600 to 1,000 m with corresponding wavelengths of about 15 km). It is almost impossible to identify the ridge crest and so its location can only be inferred from the broad belt of epicentres extending through the zone. Several fracture zones are suggested by this data but individual zones cannot be correlated with the distribution of epicentres. About 500 km from the inferred ridge crest the relief is subdued and a sequence of magnetic anomalies from 6 to 13 is recognizable. It is important to note that anomalies older than anomaly 13 are continuous across the boundary between zone A and zone B. Magnetic anomalies within about 500 km of the epicentre belt are difficult to identify and no persistent pattern can be defined in this region.

Further to the west, zone C is characterized by a well developed ridge with local relief (generally less than 200 to 300 m) more similar to zone A than zone B (Fig. 2C). The Diamantina Fracture Zone is shown near the northern edge of this profile and a minor fracture zone is indicated near the ridge crest. There is no suggestion of seismically active, transform faults with large offsets. Magnetic anomaly lineations can be traced both north and south of the ridge crest to about anomaly 19.

If allowance is made for varying water depths, the magnetic anomaly amplitudes of zone A are consistently larger than those in zone C and zone B implying a contrast in magnetic properties of the rocks from zone to zone.

The amplitudes of anomalies in zone C older than anomaly 5 are larger over the north flank than over the south flank,

and this relationship is contrary to that expected from inclination differences in the Earth's present field. The magnetic lineations and the morphology are symmetric about the ridge axis and the observed anomaly amplitude differences are, therefore, probably the result of decay (of unknown cause) of the remanent magnetization of the south flank.

The contrasts in seismic activity, inferred magnetic properties and ridge morphology demonstrate the occurrence of zones of markedly differing properties within two important plates generally considered to be internally uniform.

The locations of zone boundaries are clearly defined by the plot of "regional" depth against longitude as shown in Fig. 3. Depth variations with wavelengths < 100 km have been averaged to give the values quoted. Because the topographic "crest" for zone B is difficult to recognize, we define the "crest" as the minimum regional depth; the position of the "crest" coincides closely with the epicentre belt. There is a suggestion that the ridge crest within zone A shoals towards the east.

Fig. 4 shows the distance of a recognized magnetic anomaly⁷ from the ridge axis as a function of age taken from the geomagnetic reversal time scale⁸. Data from each zone were considered separately and different symbols used to distinguish lineations to the north of the axis from those to the south. There are several changes of slope common to all zones. If the time scale⁸ is correct, these changes reflect alterations in the rate or geometry of separation of the Antarctic and Indian plates. The time intervals between the inflexion points were examined as constant spreading rate intervals; the intervals chosen were 0 to 10 m.y., 10 to 20 m.y., 20 to 38 m.y., and 38 to 50 m.y. BP. Spreading rates (from each zone) were derived using a linear least squares analysis and are listed in Table 1. For the period 0 to 10 m.y., the crust in zone A was generated at the average half-rate of 3.69 cm yr⁻¹ and at about the same rate for both the north and south flanks. The very

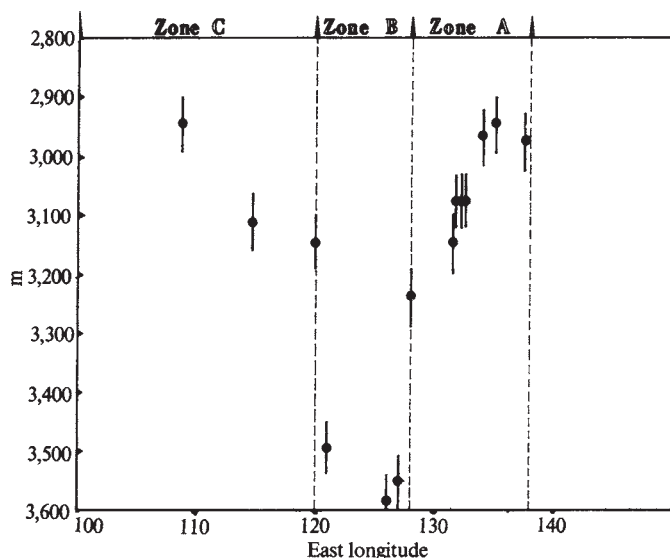


Fig. 3 Variation of minimum regional ridge depth with longitude. The estimated confidence limits are shown by the vertical bars.

limited data for the north flank of zone B indicate a relatively rapid half-rate of about 4.5 cm yr^{-1} . The spreading rate for the south flank for this period cannot be determined from these data. The spreading rate in zone C for this time interval was probably greater on the north flank than that on the south flank although the differences are only about 10%.

Table 1 Calculated Spreading Rates for Each of the Three Zones

Zone	Time m.y. BP	Half rates (cm yr^{-1})			No. of profiles	Half rates (cm yr^{-1})			No. of profiles
		North	Probable error			South	Probable error		
A	0-10	3.60	0.05		2	3.78	0.08		2
	10-20	2.85	0.06		2	2.28	0.04		2
	20-38	3.11	0.04		2	2.20	0.03		2
	> 38	2.35	0.08		2	2.57	0.16		1
	20-38	3.11	0.02		4-5	2.22	0.02		4-5
B	0-10	4.54?	0.07		2	—			
	10-20	2.35	0.06		1	—			
	20-38	2.63	0.02		1	2.54	0.05		2
	> 38	—	—		—	2.43	0.07		1
C	0-10	3.71	0.07		1	3.29	0.05		2
	10-20	2.69	0.03		1	2.41	0.04		1
	20-38	2.82	0.03		1	2.70	0.03		1
	> 38	2.38	0.02		1	2.35	0.08		1

For the interval between 10 and 20 m.y. quite good evidence exists for asymmetric seafloor spreading in zone A. There is no indication whether or not spreading was symmetrical in zone B during this time interval. In zone C there is some evidence that asymmetric spreading occurred during this period; the rate for the north flank was greater (by about 10%) than that for the south. Constant spreading rates for this time interval are not well determined for any of the zones.

The interval between 20 and 38 m.y. shows significant and systematic asymmetric spreading for zone A. The half rates are 3.11 cm yr^{-1} on the north flank and 2.22 cm yr^{-1} on the south flank. This relationship has been defined on the basis of at least four tracks over each flank and is very well documented. During this time interval zone B and zone C show approximately symmetrical spreading. The total opening rates are 5.3 cm yr^{-1} for zone A, 5.0 to 5.4 cm yr^{-1} for zone B and about 5.5 cm yr^{-1} for zone C. The small differences observed probably relate to the variation in latitudes (total maximum range 35°) with respect to the pole of opening and to inherent errors in the spreading rate determinations.

Data for the period from approximately 40 to 50 m.y. give half-spreading rates of about 2.2 to 2.4 cm yr^{-1} and seem to be generally comparable for all three zones.

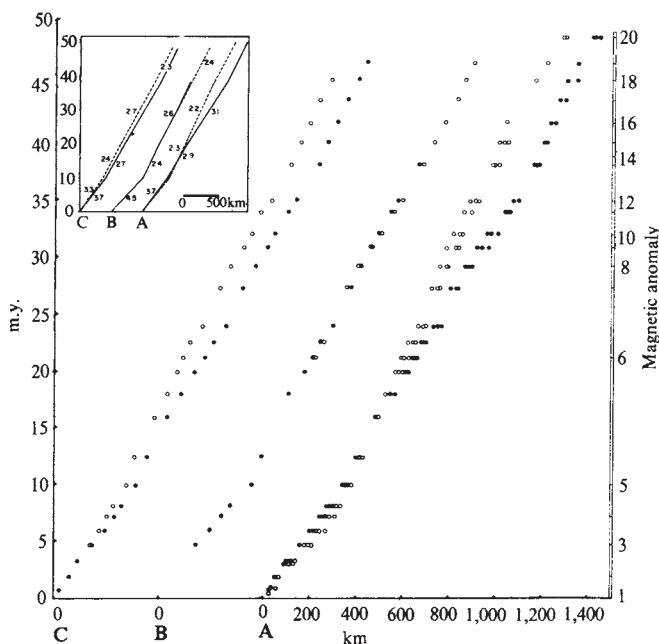


Fig. 4 Distances to key magnetic anomalies from the ridge axis as a function of age for each of the three zones. From left to right data for zone C, zone B, and zone A respectively are plotted. The distance scale is the same for all profiles although the zeroes are displaced to avoid confusion. The numbers of the standard magnetic anomalies⁷ are shown. These data were used to obtain a linear least squares fit for four assumed constant spreading rate periods. The representative spreading rates (cm yr^{-1}) are shown in the inset. The complete results of this analysis are given in Table 1. —●, North; ---○, south.

A marked difference in the distribution of anomalies in narrow longitudinal zones and a general equivalence of total separation rates lead to an important conclusion about the evolution of this accreting plate boundary. The amount of crust generated on each flank (per unit length) as determined from the inferred spreading rates is shown in Table 2. The total formation of crust for each time interval is roughly comparable for each zone although complete calculations for

Table 2 Amount of Crust Generated per Unit Length of Ridge Crest

Time: range m.y.	Zone A (km)			North	Zone B (km)			North	Zone C (km)		
	North	South	Total		North	South	Total		North	South	Total
50-38	282	306	588	—	292	?	?	286	282	568	
38-20	560	400	960	473	457	930	?	507	486	993	
10-20	285	228	513	235	—	?	?	269	241	510	
0-10	360	378	738	454	—	?	?	371	329	700	
Total 0-50	1,487	1,312	2,799					1,433	1,338	2,771	

zone B are not possible. In zone A there has been about 230 km more crust generated north of the ridge axis than south of it during the period from about 38 m.y. BP to 10 m.y. BP. The distance from anomaly 13 (about 38 m.y.) to the corresponding continental slopes of Australia and Antarctica is nearly the same (about 550 to 600 km), reaffirming that the asymmetric pattern cannot be extrapolated back to the time of initial separation. On the basis of the seismic pattern in zone B and the ridge crest morphology in zone A, we infer an offset of the ridge crest near the boundary of zones A and B of about 200 km which can be accounted for by the asymmetric spreading rates observed for zone A.

We visualize a situation in the earliest rifting history of Australia and Antarctica when there was no significant offset corresponding to the present boundary between zones A and B (Fig. 5). The present configuration of the spreading centre is partly a consequence of asymmetric spreading within zone A and symmetric spreading in the adjacent zone B. The resulting differential ridge migration leads to a progressive offset of the spreading centre and provides an explanation for the observed differences in the offset of magnetic lineations across this fracture zone boundary. It is important to realize that the observed asymmetric pattern does not require any internal deformation within the principal crustal plates. The pattern of lineations can be explained and understood in terms of evolving boundaries for each individual zone. Only the free edge or accreting boundary of the plate is affected by this differential spreading. The only condition for maintaining the continuity of the plates is that the total relative motion of the two plates is almost constant.

The contrast in the morphological properties, in the present seismicity and in the amplitudes of the magnetic anomalies—which lead us to the definition of individual zones—collectively demonstrate that these zones have evolved somewhat independently. This does not necessarily imply internal deformation of the plates. We have shown that the observed asymmetric seafloor spreading does not require internal deformation. Changes in the observed pattern of magnetic lineations, anomaly amplitudes, morphology, and seismicity are expected as a result of the motion of lithospheric plates on a sphere⁹⁻¹¹. The zonal differences observed, however, seem to define discontinuous boundaries and the discontinuities suggest at least two feasible explanations.

(1) It may be that only the accreting plate boundaries evolve in a contrasting fashion. The mechanics of crustal accretion change along the boundary between separating plates, producing zones of rough and smooth morphology, high and low anomaly amplitudes, and symmetric and asymmetric spreading patterns. For this model, the present areal extent of these features is the result of the differing boundary mechanics and of seafloor spreading. (2) The ridge morphology and the magnetic anomalies may have been generated in a similar fashion for each zone. At some time after the generation of oceanic crust and its migration away from the ridge crest, second order deformation took place within the plate, altering the thermal remanent magnetization and fracturing the ridge flank morphology. The issue of deformation within the plate is a matter of scale. If there are no subduction or accreting zones within the dimensions of the plates, then by definition first order deformation is absent and the concepts of plate tectonics hold good. This does not preclude the possibility of tectonic activity within the plate on a somewhat smaller scale.

The results of recent studies clearly demonstrate that the detailed motion of lithospheric plates about the Earth's surface requires a consideration of more than six plates¹⁰ or twenty plates⁹. Recognition of active or ancient plate boundaries may be quite difficult if the contrast in the tectonic and magnetic fabrics is subtle.

The distribution of historical seismic activity and the observed pattern of magnetic lineations can be satisfied by our first explanation.

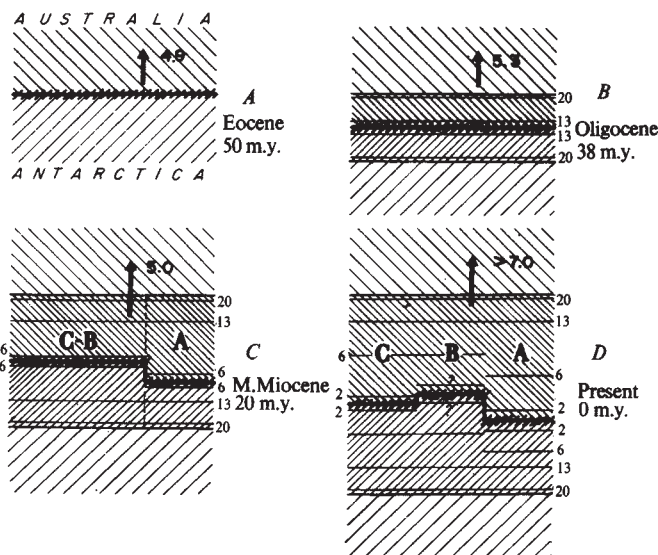


Fig. 5 Schema of the evolution of the boundary between the Indian (upper) and Antarctic (lower) plates. Assuming Antarctica is fixed: A, incipient fracturing of the continents with no offsets present along the rift; B, following symmetric spreading for 12 m.y.; C, asymmetric spreading has existed for 18 m.y. generating ridge offset as shown and differential offset of anomalies 13 to 6; D, between 20 m.y. and 0 m.y. zone C experienced asymmetric spreading generating a slight offset at the boundary between zones C and B. Anomaly 2 as shown for zone B cannot be readily identified. Total spreading rate (cm yr^{-1}) is shown near the bold arrows.

Our second explanation best explains the absence of recognizable near crestal anomalies in zone B, the variation in the amplitudes of magnetic anomalies of the same age for all zones, and the highly fractured morphology of zone B. The inferred spreading rate here is fast (half rate $\sim 4.5 \text{ cm yr}^{-1}$) in contrast to the rate expected from the topographic roughness-spreading rate correlations¹². The observed zonal differences in seafloor spreading between Australia and Antarctica probably result from a combination of both processes. The relative importance of each process has not yet been evaluated.

Zone A is the first large geographic area where systematic asymmetric spreading which persisted for a significant time ($> 20 \text{ m.y.}$) is well documented. There are many large areas of the world ocean in which only one limb of the inferred spreading system has been examined (for example, entire north-east Pacific, south-west Pacific). These areas must be reconsidered in the light of this important observation.

A detailed analysis of this entire area will be published by J. K. W. *et al.* Similarly, an analysis of asymmetric cooling at accreting zone boundaries is being considered (D. E. H., to be published) to explain the observed asymmetric spreading, and its implications for variable lineation offsets across fracture zones, generation of ridge crest offsets, and the abrupt disappearance of fracture zone morphology.

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Experiments related to Possible Production of Superheavy Elements by Proton Irradiation

MARINOV *et al.* have reported results which led them to believe that they might have found element 112, which they took to be a mercury homologue, produced by secondary reactions occurring in the irradiation of tungsten targets with 24 GeV protons¹. Evidence was presented for alpha and spontaneous fission decay associated with a species having chemical properties similar to those of mercury and a half-life of the order of months at least. As a possible mechanism for the synthesis of 112, they discussed the production, by proton scattering, of heavy recoil nuclei having kinetic energies exceeding the Coulomb barrier for their reaction with other tungsten nuclei in the target. With the assumptions that such recoils are produced with a cross section of about 1 μbarn and that they react to form the spontaneously fissioning species with a cross section about 10 μbarns , they estimated that in the 120 g cm^{-2} target, irradiated by 10^{18} protons, the yield could have been 10^3 superheavy atoms.

It was obviously of interest to attempt some independent checks on these reported observations, and two types of experiments, feasible with the proton intensities available from the Brookhaven 30 GeV accelerator, have been carried out. One experiment was designed to measure cross sections for production of very energetic heavy fragments. The second experiment was undertaken to test the possibility that the spontaneous fission might have originated, not with a superheavy species, but with an isotope of mercury or some other element which could be produced in a primary reaction of the incident protons. Thus, by use of a target of higher atomic number, Z , than that of this hypothetical species, one would be searching for a product made with a yield presumably far greater than that obtainable from secondary reactions.

The object of the first experiment was to detect, in 28.5 GeV proton irradiations, high energy massive secondary fragments which originate in a heavy element target, penetrate a layer of mylar (4.4 mg cm^{-2}), and then register tracks in a dielectric material such as silica glass. The mylar was made thick enough to absorb all ordinary fission and spallation heavy fragments and yet thin enough to transmit very energetic heavy fragments ≥ 3.0 MeV per nucleon. A schematic diagram of a typical target, absorber, and detector stack is shown in Fig. 1. In stacks used for background determination a piece of mylar was substituted for the heavy element target foil.

To achieve high sensitivity it is necessary to select absorber and track detector materials which are very pure with respect to traces of medium and heavy elements. In a trial run mica detectors were used, and the beam density was 3×10^{13} protons in $\sim 0.5 \text{ cm}^2$. After the micas were etched they were found to be saturated, in the area of the beam, with background tracks which must have originated in trace element constituents such

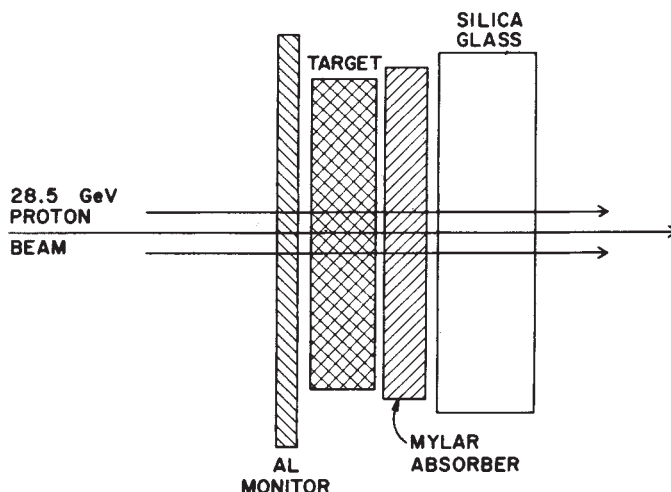


Fig. 1 Part of the stack used to search for very high energy heavy secondary fragments emitted from targets of W, Au, U irradiated by 28.5 GeV protons. Fragments must have at least 3.0 MeV per nucleon kinetic energy to penetrate the mylar absorber and record an acceptable track in the silica glass. All ordinary fission and spallation fragments are stopped by the mylar.

as iron and uranium. For the actual measurements fused silica disks were used as detectors, and although the beam intensity was increased three-fold, track densities seen in the background stacks were very low, corresponding to medium and heavy element impurity levels in the silica and mylar at least two orders of magnitude lower than in the mica.

A few preliminary test experiments were performed with the silica disks (22 mm diameter, 1.25 mm thick). They were exposed to ^{252}Cf fission fragments and etched for various times in 48% HF at room temperature. The optimum time was found to be 2.5 min, which developed the fission tracks to a diameter of 1.9 μm . Fission fragments incident at 20° or less to the surface do not produce tracks². Silica glass is about as sensitive as mica² and can record fragments with nuclear charge ≥ 16 (sulphur). Numerous background etch pits due to imperfections were observed in silica disks which were not exposed to fission fragments. Most of these pits, however, were easily distinguishable from fission tracks, and their number could be greatly reduced by annealing the silica at $1,050^\circ \text{C}$ for 16 h before irradiation.

For each of the two runs the target stacks contained six annealed silica detectors covered with mylar (4.4 mg cm^{-2}): three were placed downstream to 50 μm target foils (W, Au, and U) and three were used as blanks (Fig. 1). To bring the layers of target foils, mylar absorbers, and silica detectors into close contact they were vacuum sealed between sheets of 0.1 mm heat-sealable mylar (two units per package). In run 1 the planes of the target foils and detectors were 60° to the beam; in run 2 they were at 90° . The beam intensity and its distribution were monitored with $2.0 \text{ cm} \times 1.5 \text{ cm}$ Al foils (21 mg cm^{-2}). After each run the foil was cut into small squares and the ^{24}Na activity in each was measured with a standard proportional counter. The distribution of activity from run 1 showed that the beam was well concentrated within an area of $0.5 \text{ cm} \times 1.0 \text{ cm}$. The activity per unit area outside the beam, however, was substantial and nearly uniform; this background activity constituted 30% of the total in the beam area. It was later shown to be from scattered radiation to which the target stack had been exposed before irradiation; the stack had been in the cave near the beam for a few days before it was rotated into position for an 11 min irradiation. A correction was applied and the integrated beam intensity was found to be 1.08×10^{14} protons. In run 2 the possibility of exposure to scattered radiation was eliminated, and in 5 min the targets received a total of 0.83×10^{14} protons in an area of $0.8 \text{ cm} \times 1.6 \text{ cm}$.

After irradiation the face of one of the silica detectors not facing the beam from each run was exposed to fission fragments

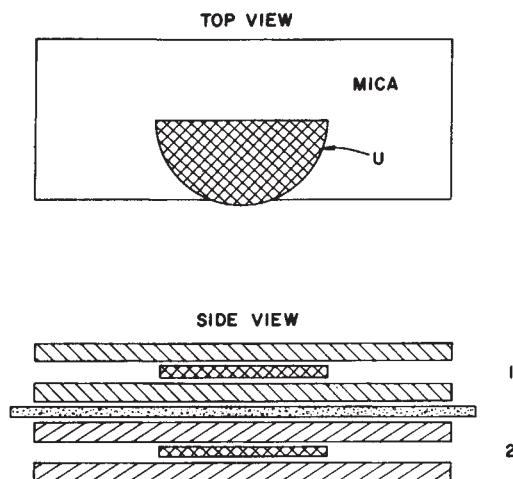


Fig. 2 Mica and foil arrangement for detection of delayed fission in targets irradiated with 28.5 GeV protons. In one run irradiated foils of U and Pb (1 and 2) were sandwiched between sheets of mica in which fission tracks can be recorded. In a second run foil 2 was an unirradiated piece of U. The two sandwiches were separated by a thin sheet of mylar.

from a ^{252}Cf source to serve as a calibration. All disks were etched in 48% HF for 2.5 min. When they were viewed in the microscope at a magnification of 200, tracks were clearly visible in the areas exposed to the beam. The density of those tracks with diameters comparable with those of fission fragments, $\geq 1.9 \mu\text{m}$ at the surface, was very low in each of the detectors; and the actual numbers of such tracks, based on duplicate scanning, are given in columns 2 and 4 of Table 1. No significant difference is seen between target silica and background silica. There were, however, numerous fine tracks in the detectors exposed to the metal targets. For U they were roughly two orders of magnitude more abundant than the larger diameter tracks; for W they were about one order of magnitude more abundant. These fine tracks will be discussed later.

Table 1 Possible Long Range Heavy Recoil Tracks in SiO_2 from Irradiation of W, Au, and U with 28.5 GeV Protons

Target	Tracks	Run 1 σ (nbarn)	Tracks	Run 2 σ (nbarn)
W	66	≤ 15	20	≤ 6
Au	46	≤ 7	—	—
U	40	≤ 5	29	≤ 10
Bkd 1	50		19	
Bkd 2	40		18	
Bkd 3	41		12	

The cross section limits, expressed in nbarns, are based on: (tracks with target) — (mean background tracks) + 2 (standard deviation). See text for explanation.

For the purpose of calculating cross section limits only the larger diameter tracks were considered; any fragments of nuclear charge ≈ 35 or greater with kinetic energies $\geq 0.4 \text{ MeV}$ per nucleon at the glass surface would produce the larger diameter tracks. Thus³, for normal incidence the fragments must have energy $\geq 3.0 \text{ MeV}$ per nucleon when they leave the metal target and enter the mylar absorber (4.4 mg cm^{-2}). At 45° the energy must be $\geq 4.0 \text{ MeV}$ per nucleon³. The minimum energy which can be important for penetrating the Coulomb barrier of heavy nuclei is taken as 5.5 MeV per nucleon. This energy then defines an effective target thickness in the U, Au, and W from which the high energy fragments, $\geq 5.5 \text{ MeV}$ per nucleon, can originate³. Only the solid angle within 45° of the normal to the surface is considered (1.84 sr). The effective thicknesses are 8 mg cm^{-2} for W and Au, 11 mg cm^{-2} for U.

Based on the observed numbers of tracks, the beam intensities, and the effective target thicknesses, cross section limits were calculated for production of very high energy massive

secondary fragments. These are shown in columns 3 and 5 of Table 1. In each case an upper limit for the net number of tracks was calculated by subtracting the mean background number and adding twice the standard deviation of the difference. The resulting cross section upper limits are all of comparable magnitude and vary from 5 to 15 nbarns. By selecting the smaller limit in each case one has: W, $\leq 6 \text{ nbarns}$; Au, $\leq 7 \text{ nbarns}$; U, $\leq 5 \text{ nbarns}$.

In the article by Marinov *et al.*¹, a cross section of $\sim 1 \mu\text{barn}$ was assumed for production of these fast secondary heavy projectiles from W, and a cross section of 10 μbarns for production of superheavy nuclei when these projectiles interact with other W target atoms. Now, using 6 nbarns as a maximum for the first reaction, it becomes necessary to assume a cross section of at least 2 mbarns for the latter reaction to account for production of superheavy nuclei in the postulated yield. It seems improbable that all the types of energetic fragments making up the 6 nbarns limit could have been effective in producing the $\approx 1,000$ atoms of the particular superheavy species reported, and thus the 2 mbarns cross section estimate should be increased at least an order of magnitude.

Nearly all of the fine tracks observed in the fused silica have much smaller diameters than those of the acceptable tracks, $\geq 1.9 \mu\text{m}$. Thus, the results shown in Table 1 are not very sensitive to the exact cut-off chosen for the track diameters. We believe the fine tracks are due to fast secondary fragments the masses and charges of which are near those of argon atoms. Nuclei of S, Cl, and Ar with energies up to 5 MeV per nucleon have been observed in scattering chamber experiments by Poskanzer, Butler, and Hyde⁴ from U irradiated by 5.5 GeV protons, and the abundance of fine tracks we observe from U is consistent with their results. Their data can be extrapolated to heavier mass fragments and to higher kinetic energy. One finds that at 20° to the beam the cross section at a given energy per nucleon of the fragments decreases one order of magnitude for an increase in Z of 3 units. Thus at 5.5 MeV per nucleon the cross sections are, in nbarns $\text{MeV}^{-1} \text{ sr}^{-1}$: 1,000 for ^{14}Si ; 100 for ^{17}Cl ; 10 for ^{20}Ca ; and 1 for ^{23}V . For tungsten targets the values are probably 10 times lower. These heavy ions may interact with U atoms to give a small yield of products in the upper actinide region and possibly even up to $Z=112$. The neutron to proton ratio of such ions, however, is very probably too low to produce those isotopes of the superheavy elements thought to be the most stable. To reach element 112 from W the projectiles must have $Z \geq 38$, and such fragments have not been observed down to the limit shown in Table 1.

The search for a spontaneously fissioning isomer⁵ which could be produced by a primary reaction of protons was made in two separate runs. In one of these a stack of Al, Pb, and U foils was irradiated for 52 min with the internal 28.5 GeV proton beam of the AGS. Total proton flux through the target, including multiple traversals, was $1.46 \times 10^{15} \text{ min}^{-1}$. After irradiation, the central active area of the stack was punched out and the lead and uranium foil pieces, half circles of 11 mm radius, were placed between pairs of mica track detectors which had been pretreated to eliminate most of the background. This pretreatment consisted of annealing followed by heavy etching in HF solution. The lead foil turned out to have been slightly damaged by heating during the irradiation; the uranium survived unscathed. Fig. 2 shows the mica-metal foil exposure arrangement. The first set of mica detectors was exposed for 2 h, beginning 20 min after the end of irradiation. A second set of micas was exposed for the next 22 h, and a third set for the following 96.3 h. The mica was etched for 90 min in 48% HF solution and examined for tracks. There were no tracks in the mica exposed to the lead foil. In mica exposed to the uranium, some tracks were observed. All were in areas adjacent to the uranium, as follows: 2 h exposure, six tracks; 22 h, four tracks; 96.3 h, twenty tracks.

Estimates based on an effective U foil thickness for fragment emission of about 5 mg cm^{-2} show that the 22 h and 96.3 h results are accounted for by spontaneous fission of ^{238}U . Two

Table 2 Fission Fragment Tracks observed in Mica Detectors exposed to 28.5 GeV Proton Irradiated U and to Adjacent Unirradiated U

Mica set	Time from end of irradiation until		Δt	No. of tracks	
	Start of exposure	End of exposure		Irrad. U	Non-irrad. U (bkd)
D	9 min	30 min	21 min	6	4
E	33 min	60 min	27 min	5	1
F	63 min	90 min	27 min	1	0
G	95 min	122 min	27 min	0	0
H*	6 h	599 h	24.7 d	152	160

* For set H, the mica-irradiated U foil sandwich was separated from the other by a distance of 70 cm. Tracks observed from this set originate from spontaneous fission of ^{238}U .

possibilities were considered for the short lived ($t_{1/2} \approx 5$ to 50 min) fragment emission observed in the first 2 h: a spontaneously fissioning species could have been produced or, alternatively, known short lived alpha emitters, which are certainly produced, could give rise, through the α, n reaction, to neutron induced fission. This would of course decay with the alpha activity.

A second similar experiment was designed to differentiate between these two possibilities. Aluminium monitor and U foils were irradiated as before but for 30 min with a proton flux, in this case, of $1.28 \times 10^{15} \text{ min}^{-1}$. Again, the punched out U half-circle was used to expose a series of mica detector pairs beginning 9 min after the end of the irradiation. This time, however, a second unirradiated piece of the same U stock and mica detectors adjacent to it were made part of the exposure stack, as shown in Fig. 2. In this way, both metal foils, being in nearly the same α, n generated neutron flux, should have had the same neutron induced fission rates, but only the irradiated foil could have been a source of fragments from a short lived spontaneously fissioning species. Tracks were observed, and again they were all in areas adjacent to the metal foils. The results, given in Table 2, show that the short lived fragment emission may well be accounted for entirely by neutron induced fission; in other words there is no clear evidence for the production, by primary reactions, of the 28 GeV protons in Pb or U, of spontaneously fissioning isomers with lifetimes greater than a few minutes. Cross section limits for production of such isomers, however, can be calculated from this experiment. If one takes the half-life of this presumed species to be 1/2 h and assumes decay by fission only, the cross section for its production would be extremely small, about 0.03 nbarns. A comparison of track counts observed for mica set H enables one to set a limit to the cross section for production of longer lived fission isomers. If the limit to the net track number observed is taken generously to be thirty-five, this cross section for a half life of 1 yr is ≤ 1 nbarn.

An indispensable contribution to this work was made by Doris Franck, who scanned the track detectors. We thank Y. Y. Chu, J. B. Cumming, G. Friedlander, E. M. Franz, P. Haustein, L. P. Remsberg, and T. Ward for discussions. This research was performed under the auspices of the US Atomic Energy Commission.

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New Technique for determining the Concentration and Distribution of Lead in Materials

So far it has not been possible to study the micro-distribution of lead in materials, except by using electron microprobes or rather crude microchemical techniques which are only really valid if the lead concentration is high.

I have devised a method for analysing trace quantities of lead in which thin sections or briquettes of compacted powders are first irradiated with α -particles of energy ~ 30 MeV. Polonium isotopes are produced from lead isotopes¹ by (α, n) reactions and α -particles emitted by the polonium are registered in sheet cellulose nitrate^{2,3} which is insensitive to the β and α active nuclides which are also produced. Table 1 shows the (α, n) reactions that are significant for the work—only the production of ^{210}Po is important because of a relatively high cross-section for capture and short half life.

Table 1 Products of (α, n) Reaction on Lead

Parent lead isotope	Reaction	Cross section for (α, n) reaction (barns)	Daughter product	Half life	α -energy (MeV)
^{208}Pb	(α, n)	1.01	^{210}Po	138 days	5.30
^{207}Pb ^{206}Pb	(α, n)	0.04	^{208}Po	2.93 yr	5.11

This technique was devised chiefly to study the distribution of lead in bone and biological tissues. There are problems associated with the dissipation of heat in the assay of dried tissues, but inorganic bone seems to be suitable when soft tissues and fat have been removed. The advantage of the technique lies in the method of registering the α -particles. The detection limit can be improved by prolonged contact of the irradiated sample with the cellulose nitrate detector. Fading of the latent track image does not seem to be significant although, for exposures of 6 months or longer, care must be taken to exclude any α -emitting contaminants.

Samples of powder were first ground to 400 mesh and then briquetted in a hydraulic press at a pressure of ~ 30 ton; thin sections of obsidian glass were cut with a diamond saw and polished to give flat surfaces. Both forms of sample were wrapped in 0.001 inch aluminium foil and irradiated on a spinning disk with the α -particles. Although the aluminium foil causes a slight degradation of beam energy, its chief purpose is to contain the sample during irradiation. For prolonged irradiation, lead-free aluminium foil is required, otherwise distillation of trace quantities of lead in the foil on the surface of the sample could result from the heat generated in the foil during the irradiation. Samples of materials of low thermal conductivity should be as thin as possible to permit efficient cooling of the sample during the irradiation. This has limited the method to the examination of samples which are unlikely to decompose or from which lead is not likely to volatilize as a result of heating. To reduce the possibility of loss of lead by vaporization, samples were restricted to a thickness of 0.5 mm and the beam current was limited to a maximum of 1.5 μA for a period of 30 min. After irradiation, the samples were set aside to allow the radioactive decay of short lived nuclides. The aluminium cover was then removed from the sample and replaced with a thin sheet (~ 1 mm) of cellulose nitrate sheet detector. The samples were then exposed to the detector in order to record sufficient α -particle tracks; for the samples reported here, an exposure period of 6 weeks was required. During the exposure, α -particles from ^{210}Po produced in the sample were registered in the plastic detector as narrow tracts of radiation-damaged plastic, the number of tracts produced being proportional to the concentration of lead (or more precisely, of ^{208}Pb) and the density of the sample. After

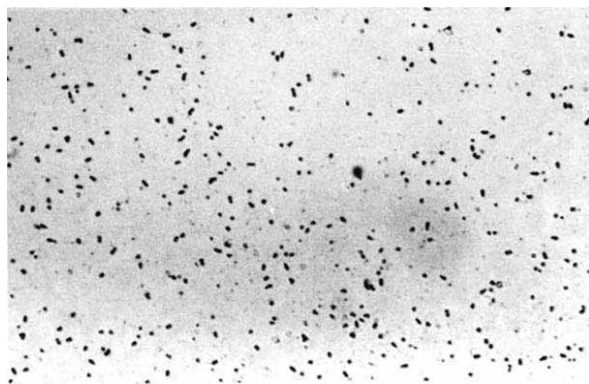


Fig. 1 The distribution of lead in obsidian glass. The small tracks have been formed in cellulose nitrate detector by the reaction $^{208}\text{Pb}(\alpha, 2n)^{210}\text{Po}$. The maximum length of track is 3 μm and the magnification $\times 140$.

suitable exposure, the detector was etched in 6.25 N NaOH for 2 min at 55°C. The etching solution preferentially dissolved away the tracks of damaged plastic to produce short tracks which could be observed using an optical microscope. The distribution of lead on the surface of the sample could then be studied in relation to the tracks produced in the detector by using a pre-aligned jig to permit accurate repositioning of sample and detector. The distribution of lead in a thin section of volcanic obsidian glass is illustrated in Fig. 1.

The concentration of lead in a sample was determined by comparing the number of tracks per unit area produced in the detector with those from standards with the same density and for which both the lead concentration and its isotopic composition were known. The isotopic composition of natural lead (^{204}Pb , ^{206}Pb , ^{207}Pb , ^{208}Pb) varies with its source and, therefore, to determine the concentration of lead by means of the reaction $^{208}\text{Pb} \rightarrow ^{210}\text{Po}$, it is necessary to know its isotopic composition. For determination of the bulk concentration of lead in the samples, $\sim 1\text{ cm}^2$ of surface was examined and more than 10^3 charged particle tracks counted to reduce the error of assay. Variations in the rate of production of ^{210}Po from lead in similar conditions of 30 MeV α -irradiation seemed to be small; the α -activity from eight 1 cm^2 samples of a lead foil irradiated for 33.75 min at 0.52 μA was 203 ± 5 c.p.s. More precise measurements can be made if the concentration of another element is used as an internal flux monitor, for example, the reaction $^{44}\text{Ca}(\alpha, p)^{47}\text{Sc}$. Although α -tracks will be produced by the reaction ^{206}Pb , $^{207}\text{Pb} \rightarrow ^{208}\text{Po}$, they are relatively unimportant; this was confirmed by α -spectrometry of the obsidian glass using surface barrier detectors. In the conditions of the experiment, the cellulose nitrate detectors were only sensitive to α -particles and so background tracks resulting from β or γ radiation were absent. If a low registration of extraneous α -events during prolonged storage or exposure is to be maintained, the detector should be sealed in a material containing no α -emitters and in an atmosphere of aged nitrogen to eliminate the radon daughter products present in normal air.

Provided that the sample can withstand the thermal effects of irradiation and that a sufficient number of charged particles are registered in the detector, the technique offers certain advantages over conventional instrumental α -particle detectors, for example, no electronic instrumentation is required for detection; background events arising from α -contamination in the detector or surroundings are very low; track formation efficiency is high in cellulose nitrate. The threshold of specific ionization for the creation of etchable tracks in cellulose nitrate is $\sim 3\text{ MeV}$ and therefore the 5.3 MeV ^{210}Po α -particles present directly on the surface of the sample are not recorded. The tracks originating from ^{210}Po in the sample therefore come from just below the surface, that is, 5.3 MeV degraded to $\sim 3\text{ MeV}$, thus overcoming the common problem of surface contamination of samples. The distribution of ^{210}Po activity on

the surface can be examined by placing an aluminium foil 0.001 inch thick between the sample and detector or alternatively a 1 cm air gap can be used; the resolution required for distribution studies is, however, then lost.

The validity of the method of quantitative assay of lead was confirmed by comparing the results obtained using the track technique with non-destructive assay for lead by a radioactivation technique. The method selected⁵⁻⁷ used $\sim 30\text{ MeV}$ electrons produced by the United Kingdom Atomic Energy Authority, Harwell, electron linear accelerator by the reaction $^{204}\text{Pb}(\gamma, n) \rightarrow ^{203}\text{Pb}$. Simultaneous irradiation of samples and standards was not possible and, therefore, to normalize irradiations cobalt oxide was mixed with the sample to act as an internal flux monitor, that is $^{59}\text{Co}(\gamma, n)^{58}\text{Co}$. Using an irradiation time of 30 min and by assay of the 0.28 MeV ^{203}Pb activity ($t_{1/2} = 52\text{ h}$) with a $3 \times 3\text{ inch}^2$ NaI (Tl) crystal, an activity of 85 c.p.m. $\mu\text{g}^{-1}\text{ Pb}$ was obtained. A comparison of the concentration of lead in various samples is given in Table 2.

Table 2 A Comparison of the Concentration of Lead by the Track and Radioactivation Techniques for Samples of Known Isotopic Composition

Material	Lead concentration (p.p.m.)	
	Track method	Radioactivation technique
Section obsidian glass	7.3	8.4
Powdered granite	15.0	16.2
Animal bone	12.2	10.8

Precision $\pm 10\%$.

Both methods are attractive for measuring the concentration of lead in samples because neither involves any chemical processing which inevitably introduces lead contamination.

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BIOLOGICAL SCIENCES

Ultrastructure of Isolated Heavy Beef Heart Mitochondria revealed by the Freeze-etching Technique

The morphology of the mitochondrion has been extensively investigated by conventional negative staining and thin section techniques. These have shown that the inner mitochondrial membrane possesses a headpiece-stalk sector¹⁻³ and that heart and liver mitochondria undergo discrete configurational changes *in vitro*^{4,5} and *in situ*⁶. Other structural and morpho-

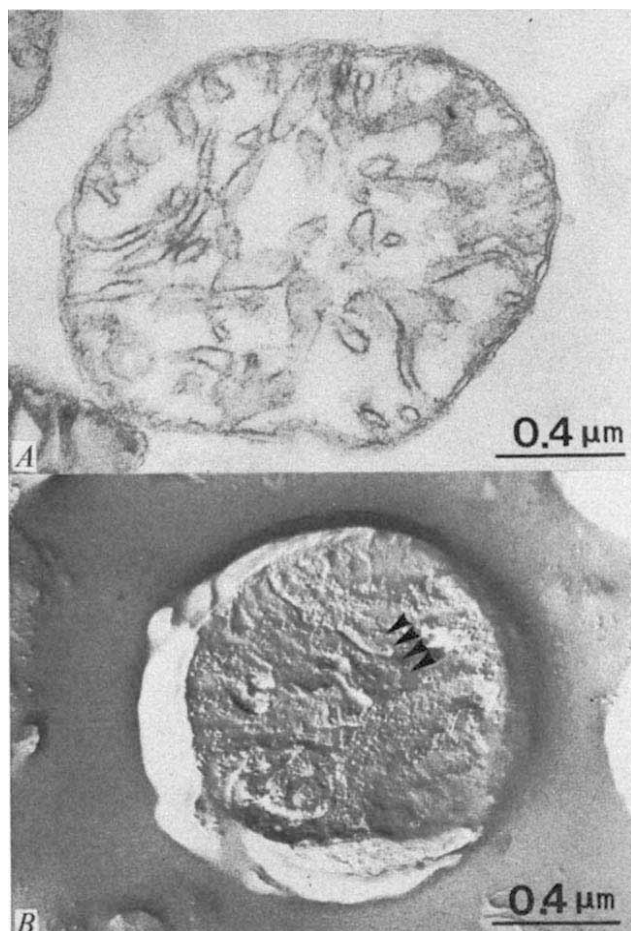


Fig. 1 *A*, Conventional thin section of a heavy beef heart mitochondrion fixed in the non-energized orthodox configurational state. Magnification $\times 40,500$. (UGDM 681.) *B*, A freeze-etched heavy beef heart mitochondrion in the non-energized orthodox configurational state. Four arrow heads indicate the direction of the platinum disposition, magnification $\times 40,500$. (UGDM 2252.)

logical features of the mitochondrial membrane have been postulated by Korman *et al.*⁷ and Sjöstrand and Barajas⁸. Here we report our observations of isolated heavy beef heart mitochondria using the freeze-etching technique.

Heavy beef heart mitochondria were isolated by the method of Hatefi and Lester⁹. They were placed in the non-energized orthodox configurational state by incubating a suspension in the following medium: 0.25 M sucrose, 10 mM Tris-Cl (pH 7.5), 2.0 mM in Tris-glutamate (pH 7.5), 2.0 mM in Tris-malate (pH 7.5), carbonyl cyanide *m*-chlorophenyl hydrozone (mCICCP), 0.1 mM; the protein concentration was 1 mg/ml. The suspension was stirred for 5 min at 27° C and the incubation was stopped by adding 50% glutaraldehyde to a final concentration of 2%. After a further 15 min incubation, the suspension was washed 3 times with a solution 0.25 M in sucrose and 50 mM in potassium cacodylate at pH 7.5.

For conventional thin sections, the suspension was stained for 60 min at 4° C with a solution 2% in osmic acid, 0.25 M in sucrose and 50 mM in potassium cacodylate (pH 7.5). The suspension was washed once with a 0.25 M solution in sucrose, 50 mM in potassium cacodylate (pH 7.5), stained for 15 min in 1% UrOAc in 25% ethanol, dehydrated in graded ethanol, washed twice with absolute propylene oxide and embedded in epon. Silver sections were cut with a diamond knife on a Sorvall MT-2 ultramicrotome, collected on 400 mesh copper grids and examined with a Philips EM 300 operated at 60 kV.

For the purpose of freeze etching, samples were washed three times in 30% dimethyl sulphoxide in water, placed in gold cups, frozen in liquid Freon 22, cut and etched in a Balzer

freeze-etching unit and examined with a Philips EM 300 operated at 60 kV. Fig. 1*A* is a conventional thin section of a heavy beef heart mitochondrion in the non-energized orthodox configurational state; Fig. 1*B* is a similar mitochondrion as it appears in a freeze-etched replica. There are numerous particles, 100–140 Å in diameter, within the matrix space. Fig. 2*A* shows a thin section of a heavy beef heart mitochondrion fixed in the non-energized aggregated state in thin section while 2*B* is the comparable freeze-etched replica. Again the particles are present in the matrix space and seem to be randomly arranged on the membrane surface. A regular arrangement may, however, be present in intact mitochondria, the particles being broken off by the cleaving process.

Fig. 3*A* shows the outer surface of the outer membrane of a mitochondrion, the fractured surface revealing the intracristal space. The outer membrane surface is rough and irregular while the outer surface of the inner membrane viewed from the intracristal space seems much smoother. Fig. 3*B* also shows the outer surface of the inner membrane. Fig. 3*C* shows an enlargement of the matrix surface of the inner membrane containing particles. We postulate that these particles could be headpiece particles containing ATPase, remembering that in freeze-etched replicas the particles will seem larger as a gloved hand will appear larger than a naked one. We have observed similar particles in the matrix space in thin sectioned material when light silver sections were examined. Experiments are now being undertaken to investi-

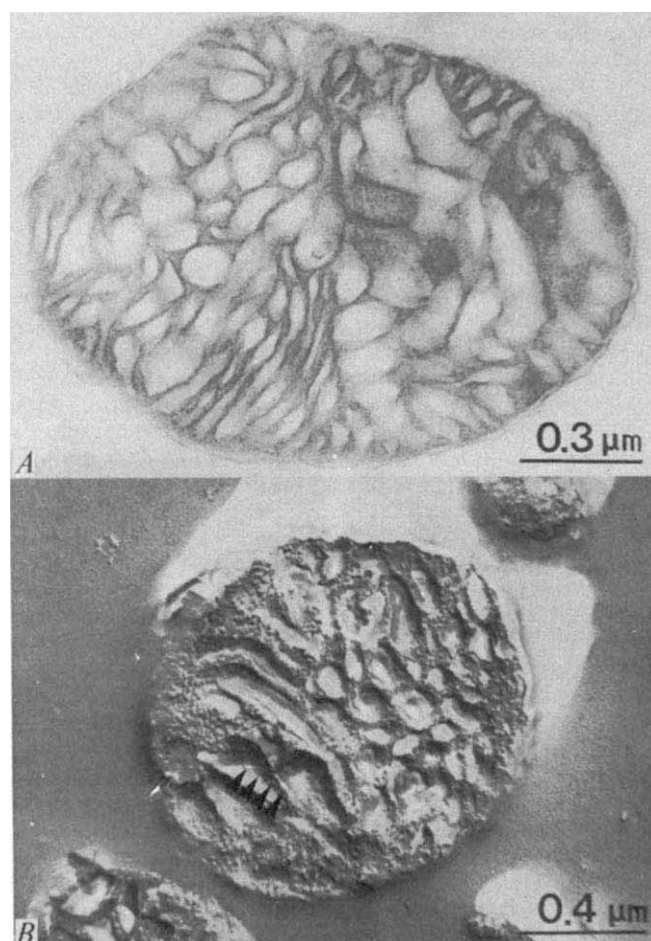


Fig. 2 *A*, Conventional thin section of a heavy beef heart mitochondrion fixed in the non-energized aggregated configurational state. In comparison with Fig 1*A*, note the decrease in the matrix space size. Magnification $\times 57,000$. (UGDM 663.) *B*, A freeze-etched heavy beef heart mitochondrion fixed in the non-energized aggregated configurational state. Note the similarity of the size of the matrix space to that in *A*. Arrows indicate the direction of the platinum disposition. Magnification $\times 40,500$. (UGDM 2265.)

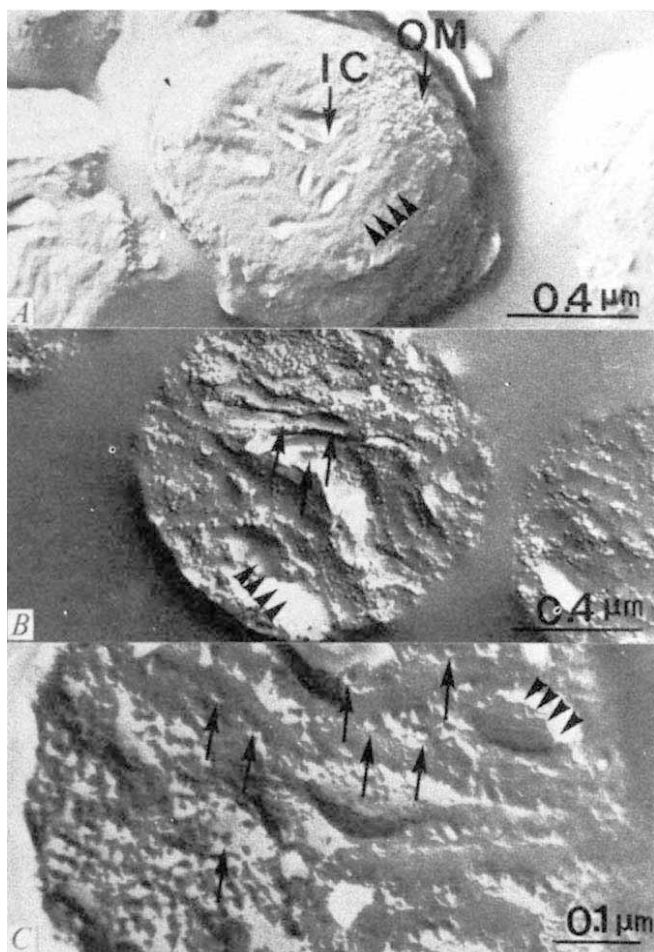


Fig. 3 *A*, A freeze-etched mitochondrion. Four arrow heads indicate the direction of the platinum disposition. Note the rough surface of the outer membrane (OM). The top of this mitochondrion has been cleaved to expose the intracristal space (IC). Magnification $\times 40,500$. (UGDM 2295.) *B*, A freeze-etched mitochondrion showing the smooth surface of the outer surface of the inner membrane; that surface exposed to the intracristal space (at arrows). ($\times 40,500$, UGDM 2257.) *C*, Enlargement of the matrix space showing numerous particles 100–150 Å in diameter covering its surface (at arrows). ($\times 117,000$, UGDM 2275.)

Electron Microscope Examination of Free IgA Molecules and of their Complexes with Antigen

THE molecules of different classes of immunoglobulin are built from units of similar structure, containing two light and two heavy polypeptide chains linked through disulphide bridges¹. The IgG molecule, which contains one such unit of molecular weight 150,000, has been shown by electron microscopy to be a flexible Y-shaped molecule^{2,3} with two arms corresponding to the Fab regions carrying the two antigen-binding sites⁴ and a third (Fc) arm. The IgM molecule consists of five Y-shaped units, each with a molecular weight of 180,000, linked cyclically by disulphide bridges through the Fc arms^{5–8}. IgA commonly occurs in the form of a bridged dimer. Such an IgA dimer preparation has been examined in the electron microscope, but the molecules shown and described had no distinct Fc feature⁹. We have examined two preparations of dimeric IgA, one isolated from the serum of mice carrying plasmacytoma MOPC 315, and the other from the serum of a patient (W.E.) with multiple myelomatosis. 10 to 15 ml. of each serum was pumped through a column (2.5 $\times$ 100 cm) of 'Sephadex G-200' (Pharmacia). Fractions were selected from the trailing side of the breakthrough peak, pooled on the basis of their antigenic purity, and concentrated by ultrafiltration (Amicon). Each preparation sedimented in the analytical ultracentrifuge as a single component with a sedimentation coefficient ($S_{20,w}$) of about 9.5S and after treatment with 5 mM dithiothreitol dissociated to the monomer of 6.3S.

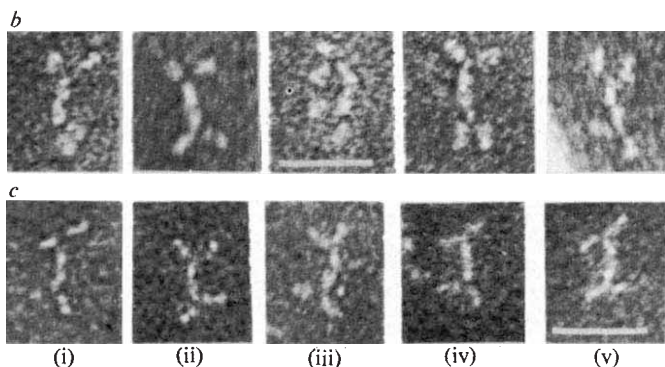
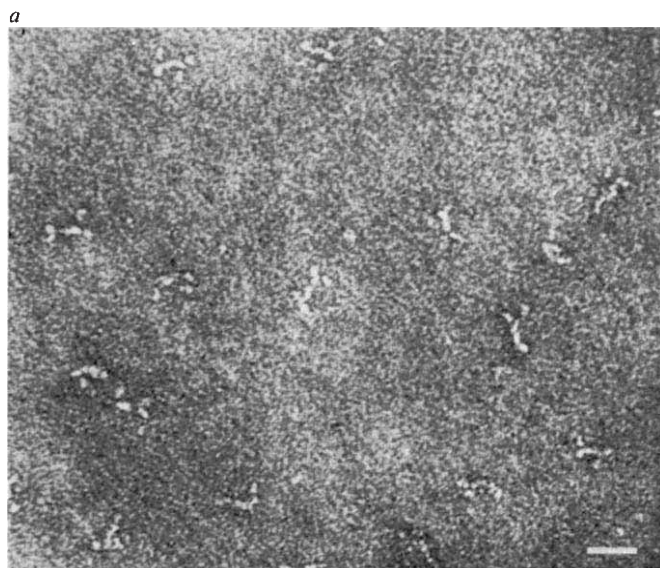


Fig. 1 Electron micrographs of free IgA dimer molecules. *a*, Human (W.E.); *b*, selected individual molecules (human, W.E.); *c*, selected individual molecules prepared from the serum of mice carrying the plasma cell tumour MOPC 315; in (iii) and (iv) the molecules have reacted with N-DNP-ε-aminocaproate. The bar represents 200 Å.

gate the nature of such particles isolated from heavy beef heart mitochondria.

We thank Dr D. E. Green for his generous gift of heavy beef heart mitochondria, and the Research Advisory Board and the Department of Microbiology, University of Guelph, for supporting this work in part.

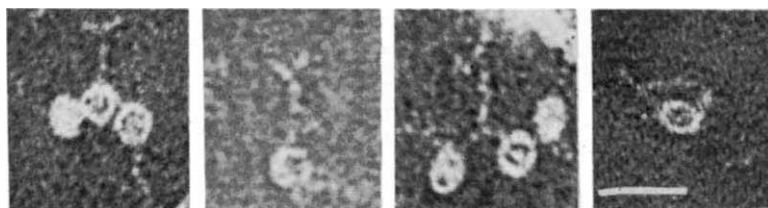
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Fig. 2 Electron micrographs of mouse IgA dimer molecules attached to DNP-ferritin. The bar represents 200 Å.



Preparations were negatively stained with ice-cold 2% sodium phosphotungstate (pH 7.3) on holey carbon films¹⁰, dried at 4° C and examined with an AEI EM6B electron microscope. The dimers seem to consist of two identical Y-shaped units, held together at the tips of one arm of each Y (Fig. 1). The length of each of the linking arms is 63 Å and the length of the free arms is 70 Å (Fig. 4). The values given are the largest found with considerable frequency. The arms are maximally 30 Å wide but vary in width, presumably because of variation in penetration by negative stain. The angle between the two linking arms is somewhat variable (Fig. 1) indicating that the molecule has a limited ability to hinge in this region. There seems to be considerably more flexibility in the "hinge" region, where the three arms of each subunit meet, judging from the variety of angles found (due allowance being made for any false impressions resulting from foreshortened views of the entire molecule). The Y-shaped IgA unit clearly corresponds to the basic four chain immunoglobulin unit.

It seemed likely that the arms linked to form dimers were the Fc regions (C-terminal halves) of the heavy chains, the other two arms of each unit being the antigen-binding Fab regions. We were able to test this possibility because the mouse protein carries binding sites for dinitrophenyl (DNP) groups on the Fab regions^{11,12}. Antigen-antibody complexes precipitated by adding the dimer to a slight excess of DNP ferritin (made with 1 mol of fluorodinitrobenzene per 5,000 daltons of ferritin) were washed free of excess IgA and examined in the electron microscope². Lattices of varying overall size were seen with IgA molecules connecting the DNP-ferritin particles. In structures such as those shown in Fig. 2 the tips of the free arms of the Ys were clearly identified as the DNP-binding sites. It is incidentally satisfactory to find that the position of the hapten binding site on this myeloma protein is analogous to the antigen binding site on the IgG and IgM subunits of conventionally induced antibody.

Mixtures in various molar ratios of the mouse dimer with the bifunctional reagents bis-N-DNP octamethylenediamine³ and the more soluble bis-DNP glycyl-lysine, and with the monofunctional N-DNP-ε-aminocaproate, were also examined. In control experiments with the DNP-ε-aminocaproate reagent the molecules were indistinguishable from the unreacted preparations (Fig. 1c iii and iv). With the bifunctional DNP reagents the dimeric IgA molecules formed lattices consisting of striking ladder-like structures linked cyclically (Fig. 3a). The overall length of the "ladders" is 120 Å and the lateral centre to centre spacing of its components varies between extremes of 32 Å and 42 Å. The cyclic nature of the lattice is a general feature of immune complexes. The double IgG links between ferritin molecules², and the rings of IgG with bis-DNP octamethylenediamine described by Valentine and Green³ are examples. Their stability is due to the relatively rapid reformation after dissociation of any link in such a cyclic complex. The ladders may themselves be collapsed cyclic structures, though their precise interpretation is conjectural. That they are formed from the Fab arms and linked to each other by Fc arms is suggested by the following observations.

The features projecting from the corners of ladders often resemble dimers the dimensions and appearance of which indicate that the two free arms of one monomer are incorporated into the ladder (Fig. 3a, inset). The ladders are frequently joined to each other by structures with dimensions and appearance which correspond to the two linking arms of

a dimer. Ladder-like structures were also obtained when monomeric IgA (obtained by selectively reducing the dimer with 2 mM dithiothreitol to produce 30% monomer followed by carboxymethylation and separation by gel filtration on Sephadex G-200) was mixed with bis-DNP-glycyl-lysine. These ladders were not linked to each other, but occasionally at the ends they carried projections comparable in size with the Fc arm (Fig. 3b). The Fc links between the monomers in a dimer evidently correspond to the links joining the ladders to each other.

Also present in the monomer complexes with bifunctional hapten are propeller-shaped structures with three or four arms of very high contrast (Fig. 3c). Again these may be collapsed cyclic structures, though the three-fold structure is difficult to reconcile with a ladder in other orientations or collapsed in a different manner.

In conclusion, the four-chain subunit of IgA closely resembles that of IgG and IgM in size, shape, position of the antigen binding site and flexibility. In the IgA dimer (shown diagrammatically in Fig. 4), two such Y-shaped units are disulphide linked through the abutted tips of the Fc arms. The geometry of this arrangement helps to explain the relative abundance of IgA dimer in IgA polymeric preparations, in contrast to the

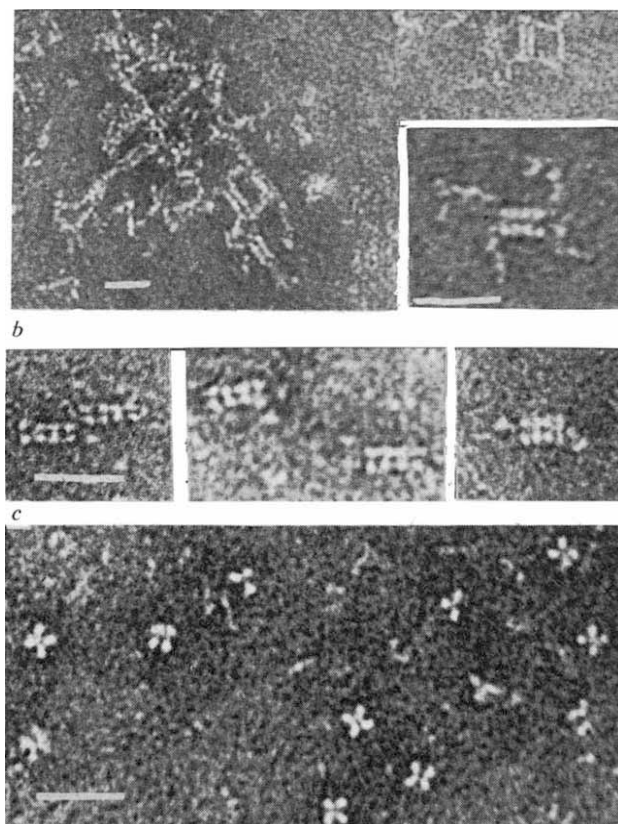


Fig. 3 Electron micrographs of mouse IgA reacted with bifunctional hapten bis-DNP-glycyl-lysine. *a*, Lattices of characteristic "ladder"-like structures made with dimer and hapten. Inset shows an individual "ladder" with material projecting from each of its four corners; *b*, selected individual ladders formed from IgA monomer-hapten complexes; *c*, an area of IgA monomer-hapten complexes showing propeller-shaped structures. The bar represents 200 Å.

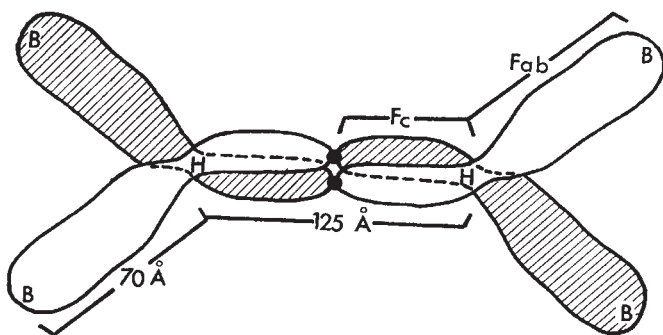


Fig. 4 Diagram of an IgA dimer molecule. The antigen binding sites are represented by B and the hinge region by H. The inter-subunit cystine bridges are shown as (●). The intrasubunit bridges are not shown. Each Y-shaped subunit is composed of two protomers, each formed from one light and one heavy chain. The two-fold axis of symmetry of each of the two subunits is aligned, giving the dimer dihedral 222 symmetry.

absence of dimers in IgM polymers, where the two heavy chains of each of the five subunits are linked to different neighbouring subunits. The IgA dimer is shown in Fig. 4 as a molecule of dihedral 222 symmetry, the monomer cysteines being so disposed that all possible intersubunit bridges can be formed. It is also possible, but less likely, that only one pair of heavy chains forms intersubunit bridges, and the second pair, although unable to bridge to each other, are sterically prevented from readily bridging to other units to form higher polymers. It remains to be established how such higher polymers of IgA are built up.

A polypeptide, J piece, has been found associated with IgA dimers¹³, but is not sufficiently characterized to allow speculation as to its role in the structure.

The similarity in appearance of the mouse and human proteins confirms that mouse and human IgA are truly analogous. An earlier report of 120,000 for the molecular weight of the MOPC 315 subunit has been more recently corrected to nearer 150,000 (Eisen, H. M., quoted in reference 12), similar to the value for the human subunit¹. It is known that in the MOPC 315 subunit the two light chains (of λ type) are bridged to each other, and that each is merely non-covalently attached to a heavy chain to form a Fab arm¹⁴. In the human molecules of subclass IgA₂ there is a similar arrangement of light chain bridges¹⁵. It is of particular interest that the human IgA dimer described here is of the other known subclass, IgA₁, where the light chains (κ chains in this instance) are bridged not to each other but to the heavy chains¹⁵. Clearly, neither the class and mode of attachment of the light chains nor the species variation of both chains substantially affects the shape of the molecule.

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Possible Teratogenic Effect of Cigarette Smoking

It is generally accepted that maternal smoking during pregnancy is associated with an increased risk of spontaneous abortion¹, low birth weight and stillbirth or neonatal death². Moreover, even at the age of 7, children of mothers who smoked in pregnancy are, on average, smaller and slightly retarded in reading ability compared with their contemporaries whose mothers did not smoke³. These associations have been shown to be independent of birth rank, maternal age and social class.

It is important to establish whether, apart from these general adverse associations, smoking in pregnancy has a teratogenic effect. Butler and Alberman² found that stillbirth and neonatal mortality rates from all congenital malformations were slightly increased in mothers who smoked, but they suggested that this might have been accounted for by the excess of mothers of high parity and low social class who smoke. It is our object here to present the results of an analysis relating smoking in pregnancy to the incidence of congenital heart disease in the population.

The data are from the 1958 British Perinatal Mortality Survey^{2,4} and its follow up study (the National Child Development Study⁵), and are in the following two parts.

(A) The control week population: questionnaires were completed for 98% of births in England, Scotland and Wales during the first week of March 1958 (17,418 questionnaires). Of these, 668 were stillborn or died within the first 4 weeks of life. Another 143 children died between 4 weeks and 7 yr of age, and for these the certified cause of death was always available and in most cases there was also a full clinical and pathological report. Ninety-two per cent of the children still resident in England, Scotland and Wales were traced at the age of 7 (ref. 3). For these a medical history was taken, and in most cases a complete medical examination was carried out, including auscultation of the heart. A firm diagnosis of congenital heart disease was accepted only when there had been a cardiac investigation, or autopsy evidence was available. In all, 106 cases of congenital heart disease were found, fifty-one having survived to 7 yr, but in our data all multiple births, and all those in whom the defect was associated with anencephalus, spina bifida or Down's syndrome, were excluded, leaving eighty-six affected infants.

(B) Three months deaths: questionnaires were also completed for stillbirths and neonatal deaths in England, Scotland and Wales during the whole of March, April and May 1958. There were 7,822 in all, most of which received a full necropsy. After excluding those with Down's syndrome and neural tube defects, as well as those already occurring in (A), there were 204 singletons with congenital heart disease.

For all the cases in (A), and the stillbirths in (B), the questionnaires were completed as soon as possible after delivery. For the neonatal deaths the questionnaire was completed after death. Distributed among social and biological questions were several on the smoking habits of the mothers, who were desig-

Table 1

Maternal smoking habit	CHD A	CHD B	Control week minus A
Smoker	34 (39.5%)	81 (39.7%)	4,626 (29.4%)
Non-smoker	52 (60.5%)	127 (60.3%)	11,093 (70.6%)
Total	86 (100%)	204 (100%)	15,719 (100%)

$A \times (\text{control} - A); \chi^2_1 = 4.2, P < 0.05.$

$B \times (\text{control} - A); \chi^2_1 = 10.2, P < 0.005.$

$(A + B) \times (\text{control} - A); \chi^2_1 = 14.3, P < 0.001.$

nated as "smokers" if they had smoked at least one cigarette a day after the fourth month of pregnancy, and had not reported a change in the average number they smoked. They were coded as non-smokers if they had not smoked at all after the fourth month. Those with changeable or unknown habits were excluded.

Table 1 shows that in both groups (A) and (B) there was a significant increase in the proportion of mothers who smoked, compared with the mothers of singletons in the control week who did not have infants with congenital heart disease. The incidence of congenital heart disease in singleton babies of mothers who smoked was 7.3 per 1,000 births, compared with the 4.7 per 1,000 of babies of non-smokers (that is, a 50% increase). Because some of this effect could have been explained by maternal age, parity and social class, an analysis of variance was carried out allowing for these factors.

For this analysis we used relevant information on the control week population and on all deaths. The cases were grouped according to whether the mother was younger than 30 or 30+; of parity less than 2 or parity 2+; of social classes⁶ I, II, III, or social classes IV, V; and whether a smoker or non-smoker.

The model $\frac{1}{2} \log \frac{x_i}{n_i - x_i} = \alpha + \beta_i$ was used, where x_i was the number of cases with congenital heart disease (CHD), $n_i - x_i$, the number of cases without CHD in a given cell, and β_i a linear function of the independent variables⁷.

The results (Table 2) indicate that the smoking effect is independent of maternal age, parity and social class, and is highly significant ($P < 0.001$).

The small number of cases in any particular category made it difficult to ascertain definitely whether any specific types of CHD were particularly associated with maternal smoking, but it seems that this may be true of patent ductus arteriosus and Fallot's tetralogy. (This finding will be discussed in a further communication.)

As far as we know, there has been only one previous study of maternal smoking habits in relation to CHD⁸, in which the maternal histories of 100 cases of CHD were compared with an equal number of controls and no significant difference was demonstrated. But an effect of the same order as we have demonstrated here is unlikely to have been statistically significant in a comparison of two samples of this size.

We think the present results sufficiently interesting to warrant further epidemiological and experimental investigations into a possible relationship between smoking and congenital heart disease.

We thank all the medical officers of health and their staffs and consultant paediatricians for their help; the Executive Com-

Table 2 Analysis of Variance

Source	χ^2_1
Smoking	11.7 ($P < 0.001$)
Parity	6.3 ($P < 0.01$)
Social class	0.6 ($P < 0.05$)
Maternal age	0.2 ($P < 0.05$)

Test for "goodness of fit" of model; χ^2 (11 d.f.) = 4.1 ($P > 0.05$).

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Reverse Transcriptases and Ageing

REVERSE transcriptases introduce new possibilities not only in tumour research but in theoretical models of ageing. It would be of immediate interest to know whether finite clones lack, and virus-transformed "immortal" clones possess, enzymes of this sort.

Ageing may be due to noise or to masking of information in the genome, or (with slightly greater experimental backing^{1,2}) to error and mis-specification at the synthetase level. Reverse transcriptases present a new feedback path to complicate such models. A reverse transcriptase would have "rejuvenatory" potential if it could restore lost or masked primary information from secondary copies. It could do this in the presence of mis-specification if it could combine information, or if it were primed only by correct RNA. If epigenetic masking plays the part suggested by Medvedev³, the price of clonal immortality in mammalian cells might be malignancy. There is also the possibility that a reverse transcriptase might be itself a mis-specified synthetase as postulated by Orgel¹. In these cases it could generate malignancy using only the cell's own genetic repertoire, appear spontaneously, but in other respects resemble a virus without viral products (other than more synthetase). Further work on the cytoplasmic transmissibility of clonal senescence and non-senescence^{2,4,5} might conceivably indicate whether any of these speculations are relevant. At the moment it appears that it is clonal senescence which is transmissible cytoplasmically and non-senescence, plus malignancy; which is virally inducible.

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Recording System for Monitoring Automaticity of Heart Cells in Culture

In recent studies with cultured beating heart cells¹⁻⁵ data have usually been acquired by intermittent microscopic observations of the contractions of the cells. The more laborious method involving measurement of intracellular electrical depolarization is too expensive for routine use⁶. We therefore set out to develop simplified culture techniques and an automated system for monitoring both the chronotropic and inotropic effects of chemical and physical agents.

We have simplified heart cell culture to provide reliable long lived, hormone-responsive cultures with up to 6 months' longevity. To automate the monitoring system we have taken advantage of the change in optical density during the contraction cycle of a cell or colony of cells. In our initial studies the amount of light capable of passing through the cells was assumed to be reasonably proportional to the degree of contraction and relaxation of the cell. Although inotropism is defined as a change in force, by measuring changes in optical density, we can measure indirectly the relative elongation and contraction of the cells during each contraction cycle, which can be related to the known inotropic effects of various drugs.

An inverted Unitron microscope (model BMIC) was modified by replacing a prism with a locally fabricated half reflecting beam splitter plate and mounting a masked photodiode in the camera port in place of the projecting lens. The change in optical density of the cells during the contraction cycle was detected by focusing the magnified image of the cells on the photodiode, amplifying the resultant voltage change, and recording the voltage on an oscillograph (Fig. 1). Thus, simultaneous viewing and recording of the contractions of the cells is possible.

The change in wave height recorded on the oscillograph was verified by replacing one of the eyepieces with an ocular micrometer and measuring the contraction and elongation of individual isolated cells, thus verifying the assumed inotropic measurement. The temperature of the culture was maintained by using an air curtain incubator. Fig. 2 shows a typical recording. Table 1 summarizes the effects of some compounds on cultured newborn rat and mouse heart ventricular cells. It is interesting that although adenosine-3',5'-cyclic phosphate has no effect on the cells, preadministration of this compound blocks the action of N⁶-2'-O-dibutyryl-adenosine-3,5-monophosphate, cyclic, suggesting that these compounds occupy common receptor sites.

Glucagon has a marked inotropic and slight chronotropic effect on cells that are adequately supplied with nutrients in

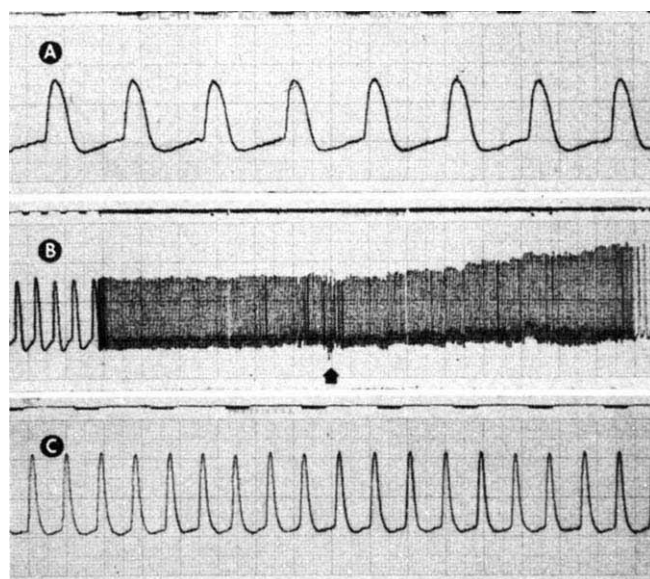


Fig. 2 Recording of the effects of adrenaline on newborn mouse heart cells after 30 days in culture. (A) Rate and amplitude recording before the addition of epinephrine. Recorder speed 20 mm/s. Contraction rate is approximately 54 beats/min. (B) Addition of adrenaline (5×10^{-8} M). Recorder speed was reduced to 0.5 mm/s in order to display amplitude increase. The arrow indicates when adrenaline was added. (C) Fifteen minutes' post addition of adrenaline. The recorder was returned to speed of 20 mm/s as in panel A. Contraction rate was approximately 126 beats/min.

fresh medium F₁₂ supplemented with 10% foetal calf serum or 10% horse serum. If F₁₂ is replaced with serum-supplemented Tyrode balanced salt solution the cells stop beating within 16 h. Replacement of Tyrode solution with fresh Tyrode solution does not restore beating nor does glucagon restore beating of cells in this depleted condition; however, within 1 h of these cells being placed in F₁₂ medium, normal beating is resumed. Cells that are in a depleted condition and are beating very slowly and weakly due to a required F₁₂ medium change can be restored to approximately normal beating rates for several days by the addition of glucagon to the depleted medium.

Table 1 Effects of some Chemical Agents on Cultured Heart Cells

Compound	Molar concentration	Activity	
		Ino-tropic	Chono-tropic
Angiotensin II	5×10^{-7} – 1×10^{-10}	ND*	+
Dichloroisoproterenol	1×10^{-5} – 2×10^{-6}	+	+
Dichloroisoproterenol	1×10^{-5} – 4×10^{-5}	–	–
Adrenaline	3×10^{-6} – 8×10^{-9}	+	+
Glucagon	6×10^{-7} – 3×10^{-10}	+	+
Guanethidine	8×10^{-5} – 2×10^{-5}	0	0
Adenosine-3',5'-cyclic phosphate	5×10^{-6} – 2×10^{-7}	0	0
N ⁶ -2'-O-dibutyryl-adenosine-3,5-monophosphate	4×10^{-6} – 2×10^{-7}	+	+
Tetraiodothyronine	2×10^{-6} – 2×10^{-7}	0	0
Triiodothyronine	2×10^{-6} – 2×10^{-7}	+	+
Tyramine	2×10^{-5} – 4×10^{-5}	0	0

* ND, Not done; +, increase; 0, no change; –, decrease.

This system provides a simplified method for monitoring and recording the direct effects of chemicals on automaticity of cardiac cells in culture. Data obtained with this system can be used as a basis for further biochemical and electro-potential studies, the sum of which could be quite useful in

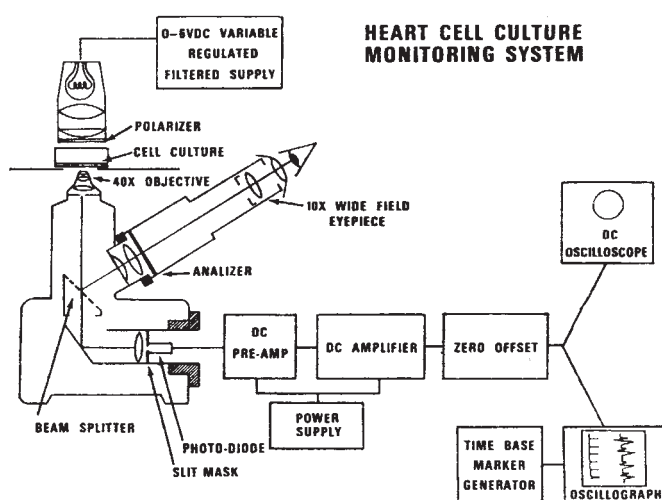


Fig. 1 Simplified schematic of the heart cell culture monitoring system.

the investigation of hormonal and pharmacological regulation of cells.

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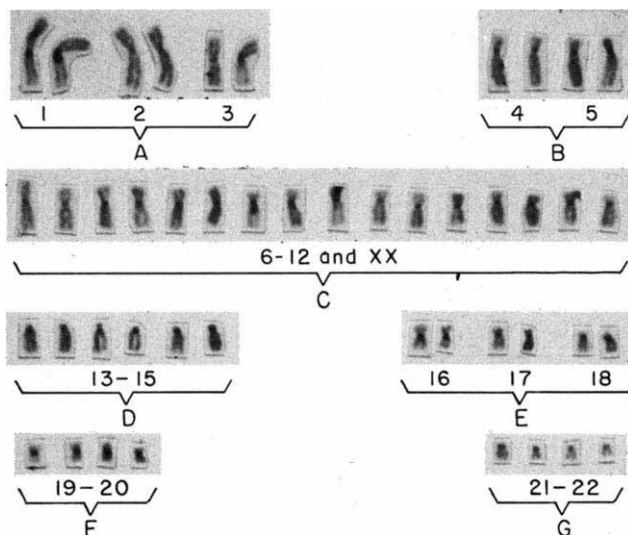


Fig. 2 Female karyotype from a human cultured leucocyte denatured and reassociated for 15 min.

Staining of Satellite DNA in Metaphase Chromosomes

A SIMPLE technique is described here which locates regions of satellite or highly repetitive DNA within mammalian metaphase chromosomes and helps the study of relationships between these regions and those which are heterochromatic and late replicating. The technique arose from attempts to hybridize radioactively labelled satellite DNA from mouse¹, guinea-pig² and calf³ *in situ* with complementary regions in metaphase chromosomes, as described by Jones⁴ and Pardue and Gall⁵. The areas of hybrids formed in this manner were always located around the centromere. Using the Giemsa stain we also observed that these areas stained more darkly than the remainder of the chromosome material on denaturation and reassociation *in situ*, without the addition of satellite DNA. This indicated that, even in these conditions, there is reassociation of the satellite DNA strands and that there may be a simpler technique in which no satellite DNA need be added. Ideally, a clear picture of the satellite DNA would be obtained in metaphase chromosomes by denaturing the DNA strands and allowing them to reassociate for varying times in an adequate buffer. In this manner, with increasing time of reassociation there would be within the chromosomes a progression of darkly staining regions of satellite DNA which would contrast

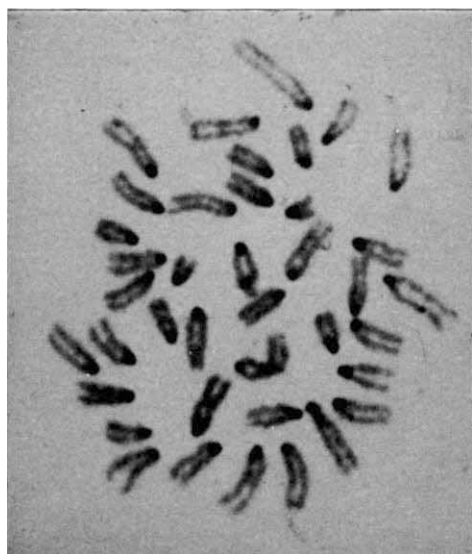


Fig. 1 Metaphase chromosomes from bone marrow of a male mouse denatured and reassociated for 30 min.

sharply with lightly staining regions of the remaining DNA.

After initial trials we chose the following reaction conditions: metaphase slide preparations of chromosomes from bone marrow cells or cultured lymphocytes were prepared by the conventional flame-dried technique, denatured for 10 min at 85–100° C in 0.06 M phosphate buffer (pH 6.8), fast cooled at 0° C, reassociated by incubation in the same buffer at 65° C for various intervals and stained with Giemsa stain⁶. When fibroblasts were used, treatment of the preparations with hydrochloric acid and RNAase before denaturation, as described by Pardue and Gall⁵, was necessary. A photograph of mouse metaphase chromosomes treated in this fashion is shown in Fig. 1. After an incubation period of 15–30 min, large darkly staining masses of reassociated DNA were observed in the areas close to the centromeres corresponding to the established areas of heterochromatin⁷ and late replicating DNA⁸. In human chromosomes at zero incubation time, no dark regions were observed; after 15 min of incubation there was reassociation of strands in small regions surrounding the centromere (Fig. 2). In male karyotypes only the distal portion of the long arms of the Y chromosome was reassociated. After 30 min of incubation there was an increase in the number and size of the areas of reassociated DNA (Fig. 3). That the pattern of reassociation is selective is suggested, for example, by the dark reassociated regions in chromosomes 4, 13, 16, 18 and 22, which are known to be late replicating^{9,10}. In contrast, chromosomes such as 15; and segments of a large number of other chromosomes, remain non-reassociated (pale) and are known to replicate earlier. A similar correlation between the regions of repetitive DNA and those of constitutive heterochromatin and late DNA replication was also established for the guinea-pig, calf, Chinese hamster and the European field vole *M. agrestis*^{11,12}. After a short period of reassociation (15 min, the chromosomes of all four species had areas of reassociation surrounding the centromere with the smallest amounts in those of Chinese hamster and *M. agrestis* and large amounts in those of guinea-pig and calf. After a longer period of reassociation (30 min), no new areas of reassociation were observed in the guinea-pig and calf, while further reassociation was observed in the giant chromosomes of *M. agrestis* and various chromosome segments of the Chinese hamster¹³. It should be noted that in all species studied, with the exception of *M. agrestis* and Chinese hamster, the Y chromosome showed appreciable reassociation of DNA strands in the distal segment of the long arms, with no reassociation in the centromeric region.

Why do the satellite DNA sequences in "fixed" chromosome preparations reassociate at rates faster than those of the bulk of DNA? In our conditions of reassociation, the DNA strands may be fully hydrated in a semi-liquid state which

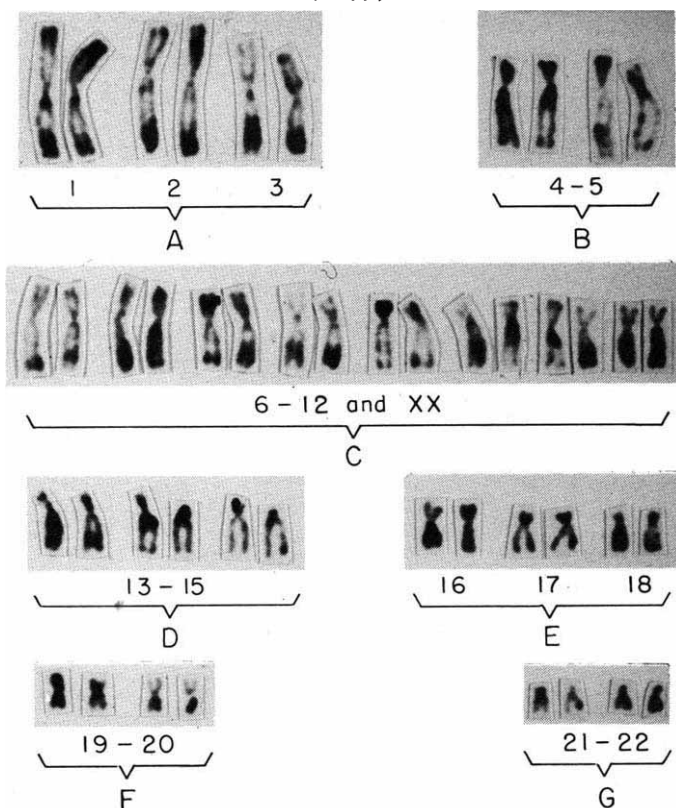


Fig. 3 Female karyotype from a human cultured leucocyte denatured and reassociated for 30 min.

permits some slipping in various regions of the DNA duplex. In these conditions, slippage to any degree in the satellite DNA regions would still cause reassociation. Since satellite DNA is composed of very short nucleotide sequences repeated a great number of times. If one also assumes that there is some degree of lateral motion of the complementary strands, and that the DNA within the metaphase chromosome is tightly packed and highly coiled and looped, especially within heterochromatin, it is conceivable that a dissociated strand from one segment of repeated sequences may reassociate with a complementary strand from another segment situated at a certain distance from it.

Modifications may be introduced to render the technique more versatile. For example, the use of formamide and/or lower ionic strength buffers may be tried to lower the temperature of denaturation and minimize heat artefacts; and in genomes where the repetitive DNA sequences are known to differ appreciably in GC content and melting temperature³, selective denaturation of one DNA species (low in GC) may be accomplished without denaturation of the other DNA species (high in GC content). This was done in the case of a calf satellite DNA which we have recently isolated from the heterochromatin of calf liver nuclei³. This DNA, which is high in GC content (55%) and has a T_m (91.4° C) six degrees higher than that of the bulk of the DNA (85.4° C), remains largely double stranded after denaturation at 87° C in 0.06 M phosphate buffer and is found as such, in the areas adjacent to centromeres after subsequent staining without reassociation. For a third modification, by the same token, repetitive DNAs which differ appreciably from the bulk of the DNA in base composition may be better visualized by pretreatment of metaphase chromosomes with agents which are known to bind preferentially to GC or AT pairs, such as Ag⁺ or Hg²⁺ ions^{2,14}.

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Block of Cerebral Actions of L-Dopa with Methyl Receptor Substances

AFTER sufficient L-dopa was administered to patients with Parkinsonism^{1,2} it was found that a large proportion of the labile methyl groups supplied in their diets was excreted as homovanillic acid (HVA)². The resulting control of Parkinsonism was sometimes marred by episodes of "freezing" while walking^{1,2}. Such episodes were reduced by co-administration of the peripheral decarboxylase inhibitor α -methyl-dopa hydrazine (MK-485) which potentiates clinical effects of L-dopa². The inhibitor has diminished, however, the proportion of L-dopa recovered as HVA in experiments to be discussed elsewhere. If HVA could block cerebral effects of L-dopa its diminution could have contributed to the potentiation of L-dopa by the inhibitor. Experimental pursuit of this hypothesis in mice led to radically different conclusions.

Tests for potential blocking of large oral doses of L-dopa have relied on counting mice falling from an opaque platform after administration of various chemicals followed by L-dopa. The platform ran inside a cage fixed 20 cm above the base. It was 7.5 cm wide, described a rectangle measuring 28 × 52 cm, and was covered with granulated corn cobs. On it were placed fifteen mice referred to hereafter as "a group".

The assay has yielded a zero blank: six groups receiving the vehicle (2% aqueous tragacanth) for all agents delivered by stomach tube remained on the platform alive for 24 h. So did animals injected intraperitoneally with water for injection, the vehicle for agents injected intraperitoneally. These tests involved more than 5,000 Swiss male albino mice of the Hale-Stoner strain, 5-6 weeks old and weighing 20-24 g. Strain was a critical variable, for the pallid (or manganese) strain³ proved very resistant to two activators of dopaminergic neurones, namely L-dopa and apomorphine^{4,5}. This resistance has not been fully overcome yet.

Hale-Stoner mice receiving graded doses of either oral L-dopa or subcutaneous apomorphine developed a dose-dependent propensity to fall (Fig. 1), due primarily to jerky and stereotyped movements which differed for each drug. All subsequent references to falling, not otherwise specified, describe straight segments of lines like those on Fig. 1. These showed correlation coefficients higher than 0.9 with at least five points representing two or more groups per point. The mortality rate from L-dopa was invariably less than the rate of falling, whereas mortality was not induced by apomorphine.

Falling and death were dissociated much better by the oral administration of MK-485 (1 mg/g mouse) 1 h before gastric instillation of L-dopa in single graded doses. The LD₅₀ of L-dopa (3 mg/g) was doubled by the inhibitor, as determined

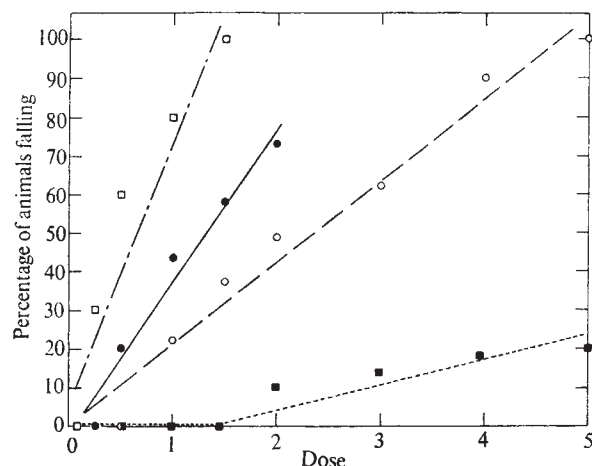


Fig. 1 Increase in falling induced by the potentiation of L-dopa with the inhibitor. ■, 0.2 mg/g of dopac+L-dopa (mg/g); ○, L-dopa (mg/g); ●, apomorphine (μg/g); □, MK-485+L-dopa (mg/g).

by probit analyses of sufficient data. The increase in falling induced by the potentiation of L-dopa with the inhibitor is shown on Fig. 1. The inhibitor alone did not seem to affect behaviour or mortality.

Intraperitoneal injection of 3-methoxy-4-hydroxyphenylacetic acid (HVA, 0.4 mg/g) into inhibitor-treated mice did not change their responses to L-dopa given orally 30 min later. Another major metabolite of L-dopa, 3,4-dihydroxyphenylacetic acid (dopac), in contrast to HVA, is an avid receptor of methyl groups, as is 3,4-dihydroxyphenylethylamine (dopamine)^{6,7}. Intraperitoneal injections of these substances (0.4 mg/g) eliminated the mortality and delayed the falling induced otherwise by L-dopa in mice treated with MK-485.

HVA was equally ineffective in blocking falling and mortality in mice receiving only oral L-dopa without MK-485. By contrast, intraperitoneal injections of dopac or dopamine (0.2 mg/g) 30 min before oral L-dopa (0.5 to 5 mg/g) grossly diminished mortality and falling (Fig. 1). Dopamine and dopac, however, failed to block the effects of apomorphine in five groups, suggesting that these metabolites did not block the dopaminergic receptors. The falling induced by apomorphine was additive to that induced by L-dopa in four groups.

Thus far, these experiments suggest that L-dopa cannot exercise its full effects on the brain if a metabolite competes for available methyl groups. Furthermore, oral administration of D,L-3-methoxy-4-hydroxyphenylalanine (3-methoxytyrosine, 6 or 8 mg/g) induced neither deaths nor falling as opposed to much smaller doses of L-dopa. The latter differs by one methyl group from 3-methoxytyrosine, as does 3-methoxy-4-hydroxyphenylethylamine (3-methoxytyramine) differ from dopamine. Methoxytyramine (0.2 mg/g), when tested against L-dopa in the range between 2 and 4 mg/g, followed the precedent of 3-methoxy-4-hydroxyphenylacetic acid by its inactivity.

If O-methylation of the catechol hydroxyl groups is indeed necessary for some actions of L-dopa or of dopamine, one should be able to block these actions with any non-toxic methyl receptor. Indeed, intraperitoneal injections of L-histidine (8) (1 mg/g) eliminated both mortality and falling caused by otherwise lethal doses of L-dopa. This large neutral amino-acid had to be injected, however, just before as well as 5 h after the administration of L-dopa, another large neutral amino-acid, otherwise both falling and mortality were merely delayed. We suspected therefore that histidine was competing with L-dopa for transport into the brain. Nicotinamide, which is not an amino-acid, can undergo rapid N-methylation⁹⁻¹¹. This vitamin was therefore injected intraperitoneally (0.5 mg/g) 30 min before the oral administration of L-dopa (3 mg/g). It eliminated both falling and mortality in four groups. This protection was duplicated by adding nicotinamide to the

animal's chow (25 mg/g) or by feeding a choline deficient diet (Nutritional Biochemicals Corp.). Both regimens were given for 25 days without inducing sedation. With both, the Hale-Stoner strain showed a resistance to L-dopa similar to that displayed spontaneously by the pallid strain.

Rates of transmethylation were not measured in any of these experiments. The methylated metabolite of nicotinamide, N'-methylnicotinamide chloride, was therefore synthesized from the vitamin in almost quantitative yields. It was characterized as will be detailed elsewhere. This analogue proved to be ineffective in preventing the fall and the mortality induced by L-dopa.

As well as preventing falling and death, nicotinamide blocked the piloerection, salivation and reddening of the paws caused by L-dopa, whereas its N-methyl analogue did not. Nicotinamide injections induced slowness and sedation—phenomena which followed the hyperactivity in animals receiving L-dopa. Sedation and slowness were also produced by dopac, dopamine and histidine but not by HVA, 3-methoxytyrosine or N'-methylnicotinamide chloride.

Table 1 Mean and s.d. of Dopamine and Dopa (μg/Brain) after Oral L-Dopa (3 mg/g Mouse)

	On platform		Off platform	
	A ₁ dopamine	B ₁ dopa	A ₂ dopamine	B ₂ dopa
Controls	0.45 ± 0.01	0.08 ± 0.01	—	—
L-Dopa	1.48 ± 0.11	0.92 ± 0.06	3.25 ± 0.67	1.69 ± 0.52
Nicotinamide (0.5 μg/g) before L-dopa	1.14 ± 0.11	1.53 ± 0.35	—	—
Dopac (0.2 μg/g) before L-dopa	0.64 ± 0.15	0.47 ± 0.08	2.08 ± 0.95	0.92 ± 0.26

Each entry represents at least seven mice.

The first mouse to fall after L-dopa was paired with an inactive mouse on the platform. In addition to the comparisons with controls, the following combinations showed $P < 0.01$: 2A₁ with 2A₂, 2A₁ with 3A₁, 2A₁ with 4A₁, 3A₁ with 2A₂, 3A₁ with 4A₁, 3A₁ with 4A₂, 4A₁ with 2A₂, 4A₁ with 4A₂, 2B₁ with 4B₁, 3B₁ with 4B₁, 4B₁ with 2B₂.

Analyses of brains for dopa and dopamine^{12,13} are still in progress but some completed observations are summarized in Table 1. They suggest that the level reached in the brain by dopamine after administration of L-dopa was a determinant in whether or not an animal fell from the platform. The production of dopamine was diminished by nicotinamide although the entry of L-dopa into the brain was apparently not impeded.

These experiments do not constitute direct evidence about the function of labile methyl groups in the cerebral action of L-dopa: the drug was given only orally and high resolution of the ensuing phenomena has not been attempted yet. The findings suggest, however, that patients should be tested to determine whether administration of methyl receptors prevents involuntary movements in Parkinsonism.

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Electron Probe X-Ray Analysis of Osmiophilic Globules as Possible Sites of Early Mineralization in Cartilage

IN OsO_4 -fixed ultrathin sections of the costo-chondral junctions of 1 month old guinea-pigs, Bonucci¹⁻³ has found osmiophilic bodies with mean diameters of 500–2,500 Å. The bodies are non-collagenous and contrast sharply with the surrounding matrix. He regarded them as the first sites of apatite nucleation, the formation of the dark needle-like crystals in the developing matrix.

To learn more about the chemical composition of these globular bodies, we analysed their calcium and phosphorus content by means of the electron microscope-microprobe analyser (EMMA) of Duncumb⁴. With this instrument, suitable fields are selected by conventional transmission electron microscopy and the electron illumination is then reduced to a probe with a diameter less than 1 µm to analyse a selected area. The measurements were made by the method used by Marshall and Hall⁵⁻⁷ for the quantitative assay of thin specimens.

Unmineralized cartilage samples from the costo-chondral junctions of 1 month old guinea-pigs were fixed for 1 h in 1% OsO_4 buffered to pH 7.4 with Veronal⁸. They were then dehydrated by treatment with 'Durcopan'⁹ and embedded in Araldite. In this way any contact with alcohol or acetone was avoided, so that only molecules soluble in water at neutral pH might be dissolved during preparation. The sections were mounted on copper grids and coated with a thin layer of evaporated carbon. Typical fields with osmiophilic globules were first found and photographed in an Elmiskop I, and the same fields were then found in the EMMA column and analysed. The EMMA instrument could not be used alone, since the limited spatial resolution of our prototype instrument would have made it difficult to find the globules and they could not have been characterized as accurately as in the Elmiskop.

The measurements were made at a column voltage of 40 kV. The calcium K_α radiation was monitored with an LiF diffracting crystal while phosphorus K_α radiation was detected with a gypsum crystal, pulse-height analysis being used to distinguish between the K_α and the second-order signal from the calcium K_β line. Continuum X-irradiation, as used in the Marshall-Hall method as a measure of total specimen mass per unit area, was recorded in the quantum-energy range 25–30 keV by means of a third detector receiving the X-rays directly from the specimen and feeding into a pulse-height analyser. The standard for quantitative analysis consisted of ultrathin sections of apatite ($\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$).

Fig. 1 shows a typical field, in which measurements were made in the encircled areas. The areas include a relatively large globule (spot 5), a group of smaller globules (spot 6),



Fig. 1 Field of view of electron microscope-microprobe analyser (EMMA) with numbered circles indicating globule containing areas analysed by the electron probe (see text).

and a control area of matrix without globules (spot 7). The circles and the ellipse formed with the aid of astigmatism represent the outlines of the electron probe, so that they delineate the microareas over which the measurements were actually made. The probe sizes were too large to contain only globules to the exclusion of all surrounding matrix; smaller electron probes could be formed but only with a reduction in probe current which would have led to insufficient X-ray intensities. Nevertheless, more than half the probed area consisted of globular material, so that the measured mass fractions may be considered to reflect the composition of the globules fairly well. The measurements plus a list of the fractions of each measured area occupied by globular material are given in Table 1.

From our results and other published observations we draw the following conclusions. First, the concentration of calcium in the globules, at least at the early stage of mineralization observed here, is not greater than in the surrounding matrix. The observed level of calcium—one or a few parts per thousand—is similar to that in similar tissues^{10,11,13}. It is much higher than the level of 0.04–0.1 parts per thousand in most soft tissues¹⁴, suggesting a process of accumulation of calcium, presumably an early stage in the process of mineralization. Second, since the observed ratio of calcium to phosphorus was often much higher than that in any calcium phosphate compound (spots 1–5), the calcium observed at this stage cannot be bound to the phosphate groups of a nucleating organic matrix. Thus the first step towards nucleation in these globules seems to be a binding of calcium on some moiety other than phosphate, in accordance with the theory of Urist¹⁵ and with our earlier results on endochondrial bone formation and cartilage mineralization^{12,16}.

It will be difficult to measure the true concentration of phosphate groups in the globules in the early stage of mineral-

Table 1 Measurements of Calcium and Phosphorus Mass Fractions

Spot	Nature of field	Globular fraction (%)	Mass fractions (%)	
			Ca	P
1	Several globules	75	0.08	not detected
2	Several globules	75	0.13	0.03
3	Several globules	40	0.23	0.03
4	Several globules	40	0.42	0.04
5	One globule	50	0.23	not detected
6	Several globules	25	0.22	0.15
7	No globules (control)	0	0.29	0.09
8	One globule	35	0.14	0.09
9	Several globules	70	0.14	0.14
10	No globules (control)	0	0.20	0.18

Each measured mass fraction is the ratio of the mass of the assayed element to the total mass of specimen present in the probed micro-volume. In referring to "the specimen" we do not include the 'Formvar' supporting film, since its mass is monitored in a separate background observation and is subtracted in the calculations, but the specimen mass does include any embedding material which may be present in the probed volume. The tabulated mass fractions therefore refer to tissue plus embedding medium, and are equal to or somewhat less than the mass fractions in the dried biological tissue proper.

Measurements 1-4 were carried out in one set of sections and 5-10 in another. There is a difference between the two sets in the average calcium/phosphorus ratio, probably due to different degrees of loss of phosphorus in the course of specimen preparation. It has been shown elsewhere that much higher phosphorus concentrations are observed in the pre-mineralized organic matrix when dry preparative techniques are used to avoid loss, while variable losses of phosphorus occur in wet preparations¹⁰⁻¹².

ization which is being considered here, since OsO₄ solutions are used to render the globules visible but the water of these solutions seems to remove the phosphate groups. Fixation with OsO₄ vapour may be a way of overcoming this difficulty.

We thank the staff of the Tube Investments Research Laboratories, Saffron Walden, England, in particular Dr P. Duncumb, Dr C. J. Cooke and Mr P. Hunneyball, for the use of their electron microscope-microprobe analyser.

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Cholera and Prostaglandins

CHOLERA exotoxin and prostaglandin E₁ have a similar effect on electrolytes in rabbit isolated ileal mucosa^{1,2}. Application of either cholera exotoxin or prostaglandin E₁ (0.1 mM) to the mucosa inhibits sodium absorption and stimulates chloride secretion. Furthermore, prostaglandin infused into the superior mesenteric artery in dogs causes loss of fluid into the intestine¹. Greenough *et al.*¹ suggested that both the toxin and the prostaglandin E₁ act on cyclic adenosine monophosphate, because the responses are mimicked by the phosphodiesterase inhibitor theophylline. The similarity of effect of cholera exotoxin and prostaglandin E₁ extends to man. A characteristic of cholera is the profuse watery diarrhoea, and cholera toxin and theophylline inhibit sodium absorption and stimulate chloride secretion in human isolated ileal mucosa. Prostaglandin E₁ given orally to man causes diarrhoea^{3,4} which was described⁴ as normally formed faeces in clear fluid which suggests an effect on intestinal secretion.

One possible explanation for the effect of cholera toxin on secretion is that it stimulates the release or synthesis of prostaglandin which then acts on cyclic adenosine monophosphate; E-type prostaglandin occurs in the human intestine (unpublished results of A. B., I. F. Stamford and W. G. Unger), and synthesis of prostaglandin has been shown in the animal gastro-intestinal tract^{5,6}. The involvement of prostaglandins could be tested *in vitro* with prostaglandin antagonists^{7,8} but there are not yet available for administration to man. Vane and his colleagues⁹⁻¹¹ have shown that acidic anti-inflammatory drugs such as indomethacin and aspirin inhibit the synthesis of prostaglandins *in vitro* and they suggest that some therapeutic effects are due to this inhibition. The possibility that cholera toxin stimulates prostaglandin synthesis could be tested safely and quickly in the epidemic in India; indomethacin or aspirin could be administered to determine the effect on diarrhoea. A negative finding would be of value, but a positive result would be of enormous benefit in the prevention of dehydration and the resultant suffering and death.

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BOOK REVIEWS

Beyond Alchemy

A History of Chemistry. Vol. I, Part 1: Theoretical Background. By the late J. R. Partington. Pp. xiv + 370. (Macmillan: London; St Martin's: New York, December 1970.) £10.

WE all know that Partington did not finish preparing his great work for the press and that the material which should have made up Part 2 of Volume I still presents editorial problems of great complexity. So we are in the curious position of having Vol. I, Part 1, and three other volumes (II, III and IV). The latter begin where it was often convenient to begin the history of chemistry, that is to say with the technological treatises of the 16th century in the ascendant and with alchemy in disreputable decline. If we had only these three we should still be rich. For all its exasperating inconsistency and its disorderly array of irrelevance it is a marvellous quarry of material. A quarry, however, not an edifice. *De mortuis*: but one must be critical. Partington was apt to censure others for being unhistorical when they filled gaps in knowledge with imaginative inference. But he was open to the charge of refusing to indicate how inference might be guided. Superficially he did the historian's first duty, the duty to narrate, but he never really learned to tell an extended story. His Presidential Address of 1951 to the British Society for the History of Science, "Chemistry as Rationalized Alchemy", which is printed as a preface to Vol. I, Part 1, is, indeed, an attempt to condense the history of chemistry in a single *pièce d'occasion*, but is little more than a succession of comments on individuals.

The merit of the *History of Chemistry* lies not in its form, which is a glorious muddle, but in its bulk. Clearly, it was put together by the accumulation of notes made over many years, with each paragraph edited to give it some balance, but often with little connective matter to help guide the reader. Ask what Plato or Aristotle thought about water and you get long answers. Ask how the idea of the nature of water changed and one has to make up an answer out of dozens of bits and pieces. But this is to criticize Partington for what he asserted he was doing. If we look at what he really did it looks different.

This is, in fact, no history but a kind of encyclopaedia arranged by authors, in rough chronological order, so detailed in its summation of the work of each as to have also something of the character of an anthology. Recognizing this can give us some relief and encouragement. A real history of

chemistry would be so enormous as to leave little room for anything to grow in its shade. It is instructive, however, to read parallel passages of Sarton's *History of Science* (the late work, not the Introduction). Lucretius, for example, is dismissed as a person by Partington in a dozen lines, as an accurate source for the doctrine of Epicurus, which is then dealt with at length. Sarton elicits from the character of the poem not only a portrait of the poet but also an evocation of the significance of Epicurean atomism for our understanding of the scientific thought of the time. Sarton is deficient in fact, Partington deficient in insight. Obviously there is room here for a substantial twentieth century appreciation of Lucretian Epicureanism, but, more to the point for the ordinary student, no one need feel that he must be overawed by the existence of earlier work from having a go on his own at big topics.

Looked at in this way the value of a great part of this volume is clear. Anyone wanting to tackle any aspect of the history of chemistry from the early Greeks onwards will find here usually a summary, and sometimes a complete list, of the main ideas and assertions of every relevant author.

But if the history of chemistry is a history of ideas about the nature and meaning of matter, do we not need to see the development of ideas in terms of the common and individual experiences which the philosophers thought it necessary to interpret? Partington examines nothing and relates nothing. One begins to suspect that he had no faculty for deciding on the relative value of material. If he had, would he not have given some introduction to the last chapters on the mystery religions? We need some modern study of their relation to both alchemical and non-alchemical ideas in chemistry. It is difficult to see from Partington how Manichaeism, Gnosticism or Mithraism contributed to the philosophy of matter, let alone chemistry, from these long lists of purely religious or bibliographical observations, often culled, as in the case of the Sabians, from rather few secondary sources.

We are left with no real beginning to our history of chemistry. Anyone who wants real origins had better turn to Multhau. But for all its shortcomings, the still incomplete Partington is bigger and fuller than anything else we have or are likely to have for some generations. So we shall reluctantly pay the high price for this latest part because we cannot do without it.

FRANK GREENAWAY

Medicine Morals

Science and Morality in Medicine: a Survey of Medical Educators. By Earl R. Babbie. Pp. xviii + 261. (University of California: Berkeley, Los Angeles, and London, 1970.) \$7.50.

THERE was a time when the chief function of the physician was to prevent his patient from behaving foolishly and to give him moral support in case of illness. Little was understood of the process of disease and little could be done to alter their course and outcome. The growth of scientific knowledge and the development of a technology based on science has altered all that. Medicine has succeeded in altering the whole structure of society and its outlook. The Indian sub-continent is rapidly progressing to a state of such over population that the mind boggles at the probable outcome. The wealthier societies become top heavy with aged pensioners.

Medical care is seen by many as an elementary human right. Irrespective of race, colour or creed, all should have access to it. Unfortunately the facts do not admit of this. When the practice of medicine has become so refined an art as it is today, the difference between the best and the worst, between the most able and the best trained and the less able and the worst trained, is enormous. Medicine has in the past been a profession in which distinction has carried the privileges of fame, position and wealth. The best achieve this simply by excellence, but there have always been others who achieved wealth by less admirable means. These are some of the factors lying behind the present situation in the United States. The American Medical Association has fought against what it calls socialized medicine and for free enterprise in medicine. Its success has gained great wealth for the profession but it has not been quite so happy for the patient.

When things go wrong, or seem to go wrong, it is customary to find a scapegoat. Science is the first choice of the contemporary world. It is said that the decline of morality in medicine is due to corrosion by science and scientists. To find out by sociological methods, or methodology, as the author would put it (long words are scientific), is the purpose of this book. A questionnaire (15 pages in length) of 67 questions was sent to 627 full-time faculty members in medicine and paediatrics at 12 of the nation's medical colleges. 454 (72%) completed the questionnaire. How different was the response of part-time faculty members; only 17% completed theirs. No doubt they were busy attend-

ing the sick. Scientific orientation was measured by answers to questions which showed "(a) their ultimate interests in problems of understanding basic mechanisms rather than total patient management, (b) their greater interest in literature dealing with the basic rationale underlying new therapeutic treatments rather than the effectiveness of those treatments, and (c) their self identification as researchers rather than as practising physicians. Faculty responses to each of these items were highly inter-correlated, and a composite index of orientations was constructed from them." Answers to further questions led Babbie to conclude "that science does not undermine the traditional norms of humane patient-physician relations". Looking for the basis of morality, he found religion and science were incompatible. "Moreover, Catholicism and Protestantism—hypothetized as the most involved in the free-will image—produced fewer scientific orientations than did Judaism or agnosticism."

Babbie was surprised (I would not have been) that views of moral questions were formed more by social than medical attitudes. The right to medical care, for example, was not correlated with a physician's knowledge of practical medicine. Attitudes to infanticide were most closely correlated with religion.

This is an informative book on an interesting subject, but why do social scientists, particularly American social scientists, murder the English language?

GEORGE PICKERING

Theology and Science

William Dell: Master Puritan. By Eric C. Walker. Pp. x+238. (Heffer: Cambridge, 1970.) £3.

WILLIAM DELL, who receives only passing notice from historians of science, was one of the religious fanatics denounced by the two celebrated Oxford founders of the Royal Society, John Wilkins and Seth Ward. Their often cited *Vindiciae Academiarum* (1654) consigned Dell and other university critics to an oblivion from which they have only slowly re-emerged. Before this present book was published, the reader had access to no reliable accounts of the life and works of Dell. The patient and meticulous labours of E. C. Walker have repaired this neglect and have provided one of the best studies of puritan intellectual life in the seventeenth century.

From our point of view, Dell is more interesting and enlightened than previously recognized. His religious opinions necessitated a policy of extreme secularization of university studies.

This was a highly unpopular and somewhat paradoxical position for a clergyman head of Caius College, Cambridge. But Dell remained an uncompromising critic of his colleagues, concluding that the sciences were a theologically innocuous and economically valuable foundation for education. Equally novel was his advocacy of the great extension of higher education, which should be adapted to local economic needs. Thus Dell appears as a lone proponent of scientific education in an age when even the founders of the Royal Society were complacent about the adequacy of scholastic studies. Dell was quickly ejected from his university offices, his ideas being abandoned until the nineteenth century, when it was again possible for the development of secularized and scientific university education.

C. WEBSTER

Energy Budget

Productivity of Terrestrial Animals: Principles and Methods. By K. Petruszewicz and A. Macfadyen. (IBP Handbook No. 13.) Pp. xii+190. (Blackwell Scientific: Oxford and Edinburgh, 1970.) £2.50.

THIS is one of a series of handbooks prepared for the guidance of research workers in the International Biological Programme. Although the operational phase of IBP draws to a close in 1972, studies of the productivity of terrestrial animals will continue so that the usefulness of this book will long outlive its immediate purpose.

As well as stimulating ecological research, the IBP has been valuable in promoting the international exchange of ideas and information. The present work is a good example of this; English-speaking readers will, for example, find great value in the presentation of the ideas and methods of the outstanding Polish workers and no doubt the reverse will be equally true.

The IBP handbooks are intended to give workers in different parts of the world a methodology for research whereby their results can be compared. This is clearly difficult unless the treatise is confined to well established and thus sometimes outdated approaches. Fortunately the authors avoid the obvious pitfalls of producing a set of standard recipes and, on the contrary, the reader is left with an impression of production ecology as a field in which concepts and methods are both numerous and flexible. This is as it should be in a science still young.

The book takes one from its definitions of terms and units of measurement of metabolic balance in animal populations to a consideration

of the theoretical basis and practical devices for estimating the parameters of an energy budget. Having absorbed the usually detailed and only occasionally tortuous discussion, the worker will have a good idea of the kinds of measurements required for the construction of an energy balance sheet for an animal population, and will be well aware of the problems involved in integrating the separate measurements involved. The mathematical formulations given for the calculation of production are not too daunting and appear to be workable with no more than the usual mathematical equipment of the majority of ecologists. The practical details given of the various relevant techniques such as respirometry and calorimetry will at least enable the reader to identify those most appropriate for a particular study.

No doubt the authors were faced with a difficult choice between writing a good book or a good handbook. We must be grateful that they chose the former with the result that their exposition will provide an invaluable, but not inflexible, source of fact and inspiration for many years.

F. B. O'CONNOR

Igneous Africa

Symposium on the Bushveld Igneous Complex and other Layered Intrusions. (Special Publication No. 1.) Pp. xii+763. (Geological Society of South Africa: Johannesburg, 1970.)

THIS massive volume contains the papers contributed to a symposium on the Bushveld and other layered basic intrusions held at Pretoria in July 1969. The Geological Society of South Africa, and its editors, are to be congratulated on getting this very large volume of new work published so quickly. As the first of the society's series of special publications, it sets a very high standard. It is also a fine memorial to the late Professor Willemse, who originally planned the symposium.

The Bushveld Complex is a layered basic intrusion nearly 2,000 million years old. Covering an area of over 60,000 km², it is the largest such intrusion in the world and it contains correspondingly unusual resources of chromite, platinum and other valuable minerals. It remains in many respects an enigma. A great deal of detailed research has been done on the complex recently, and it was timely to have the new data presented, discussed by a gathering of international experts and published.

The resulting volume serves as a complement to Wager and Brown's masterly work on Layered Igneous Rocks, by providing a mass of new

data, not only in the first half of the volume, about the Bushveld Complex, but also about other layered complexes in southern Africa and elsewhere in the world which throw light on the problems of the Bushveld Complex.

The way in which an injection of silicate melt, several kilometres thick, crystallized so as to form the extraordinarily regularly stratified and often sharply defined layers of the different minerals seen in the Bushveld is explored in many of the papers. This can only be understood when the progressive changes in composition of the component mineral phases, from the floor of the intrusion to its roof, are accurately known. A wealth of such data is provided, by Coertze for the Western Bushveld, de Villiers for the district south of Potgietersrus, Croeneveld for the Stoffberg area, Cameron and von Gruenewaldt for the eastern Bushveld, Fergusson and Wright for the Critical Series and van Zyl for the Merensky Reef. There are also many new data on the variations in the proportions of the various minerals upwards through the complex and on variations in bulk chemistry of the rocks. Liebenberg contributes a major paper on the sulphide minerals. There are also some new data on textures and structures. All this information could be synthesized, using the principles which were first worked out by Wager and Deer from their study of the Skaergaard intrusion in East Greenland, to reach a clearer understanding of the far more complicated Bushveld intrusion.

But no such synthesis is attempted in this volume. The various contributors express various views, some finding evidence that the melt was injected in successive heaves, while others believe that the sudden changes in the composition of the crystals that were accumulating can be explained by variations in pressure and water content or by variations in oxygen fugacity. Nor are the differences in interpretation clarified by the reported discussions, which are brief and almost perfunctory. It would have helped the reader if someone had attempted to assess the implications of all the new work that was presented.

The geometrical form of the Bushveld Complex remains problematical. The clearest advance in precision, reported by Biesheuvel, came from gravimetric work, supported by deep drilling, in the western Bushveld. In many areas, faulting has had more effect than had generally been realized. Discussing the structure of the Marble Hall area, de Waal shows that the distribution of the metasediments can be interpreted in terms of interference folds. This Marble Hall area is, however, one of the uplifts which Hamilton, in a provocative and admittedly specu-

lative paper, takes to mark the site of one of three simultaneous hypervelocity meteorite impacts to which he attributes the melt from which the Bushveld Complex crystallized. He naturally welcomed Labuschagne's announcement, in the discussion, that he had discovered shock metamorphic lamellae in the Rooiberg quartzite in the roof of the Bushveld Complex.

The papers on other layered intrusions form a less coherent but no less interesting group. Thayer emphasizes the distinction between stratiform and podiform chromitites; most of the economically significant stratiform bodies are in Pre-Cambrian rocks and the podiform ones in Palaeozoic or Mesozoic rocks. But the reality of the distinction between the stratiform and Alpine types of layered intrusion was not supported by the data presented by some other contributors. Van der Kaaden, in an interesting review of the chromite-bearing ultramafic and related rocks in Turkey, emphatically rejected Dubertret's claim that there is a gradation from ultramafic to submarine lavas. He preferred to interpret these ultramafic rocks, as Gass interpreted the Troodos Massif in Cyprus, as slices thrust up from the mantle.

Davies, Allsopp, Erlank and Manton present and discuss strontium isotope data on the Bushveld and other southern African layered intrusions. The ages range from $1,372 \pm 142$ million years (Trompsburg) to $2,874 \pm 30$ million years (Usushwana); the Bushveld and Losberg ages are indistinguishable; the Great Dyke of Rhodesia is much older. The acid rocks of the Bushveld Complex probably represent crustal rocks remelted at depth.

The thirty-eight contributions have been well arranged; printing errors are rather numerous but mostly trivial. The ornaments on two of the main formations on a map on page 339 have been interchanged. The choice of ornament for the maps is generally bad, with the result that they are difficult to read. Despite these blemishes, the volume is a most important one which will be widely used by geologists concerned with igneous, economic and African geology. R. M. SHACKLETON

Teaching Geology

The Earth: an Introduction to Physical Geology. By John Verhoogen, Francis J. Turner, Lionel E. Weiss and William S. Fyfe. Pp. vii+748. (Holt, Rinehart and Winston: New York and London, 1970.) £7.20.

WE continue to hear about the problems connected with the scientific image of geology. Various suggestions have been made and continue to be made about the ways of improving the image of

geology as presented to laymen, members of parliament or congressmen, industrial corporations and students. In the long run, the most important group amongst the above categories is the student, for it is the student of today who will tomorrow belong to the other categories. And the best way to present geology to a student is to provide him with the very best of textbooks which will show how exciting modern geology has become. A recent trend has been to publish the highlights of modern geology and invariably these have become smaller geophysical tracts or monographs which are easy and interesting to read but one would be hard pressed to call them textbooks with great depth. They serve a useful purpose in that they attract the first attention of the student but they are not the kind that one grows with.

The present volume, however, is the kind that one keeps going back to throughout one's undergraduate days and, even if one "picks" at it in different places, one gets a full morsel that will survive the three or four years of college. I find it hard to be calm and dispassionate about this book since there just is not another book like this in geology today. In the preface to the book the authors have squarely put geophysics and geochemistry where they belong, as "... the more analytical branches of geology". By incorporating physics (elementary but including vectors, tensors and matrices, and at the same time explaining them) and chemistry (including elementary chemical thermodynamics and crystal field theory) throughout the book, the authors have valiantly, and successfully, fought off the idea that geology is mainly "a stamp collecting type of science", in the sense in which Lord Rutherford used the phrase.

As a teacher of seniors (third year honour students in Britain) and graduate students, I welcome this book as a tremendous preparatory text. There will surely be need for specialized texts in geophysics and geochemistry, but, the student having been brought up on a book like this in the earlier undergraduate years, there will be no need for the teacher to talk down to him, eager for a specialized training in geophysics or geochemistry. The illustrations are numerous and good—the technique of using one other colour, in addition to black on white, has paid off. An example is Fig. 3-73 which is C. R. Allen's composite map of four of the world's major transcurrent faults. The same figure is reproduced as Fig. 7.1 in F. D. Stacey's *Physics of the Earth*, but the clarity of the figure in the present volume is obviously superior because of the use of a single additional colour. In spite of the modern nature of the book—which I like so much—it should

be pointed out that the chapters follow very much the classical line of geological thought. Thus, the reader begins by looking at things nearest to him—minerals, geological structures and so on—then descends into the mantle to find explanations for the surface observations and only then is led to the ideas about the chemical evolution of the Earth. This may not be to the taste of the committed modernist, but would be more acceptable to geologists in general. The structural geology laboratory instructor would be pleased to note a valuable appendix on how to represent structures in two dimensions.

One cheerful quality of the book is its healthy scepticism about many hypotheses—thus there is always an attempt to delineate the limitations of a hypothesis, however comprehensive it may appear to be; an excellent exercise in epistemology and, heaven knows, one needs that in all the Earth's sciences. For the student of printer's devil, there are some interesting examples, thus "S. California" for "S. Africa" in the title of Fig. 3-63 (the book was written in Berkeley, California) and "Fujiyama" instead of "Fujisawa" on p. 657. But these are so few and far between as to constitute merely amusing interludes in an otherwise excellent and highly recommended textbook for undergraduates, and graduate students, of the Earth sciences. SUBIR BANERJEE

Ecology by Computer

Atlas of Energy Budgets of Plant Leaves. By David M. Gates and LaVerne E. Papian. Pp. vii+277. (Academic: New York and London, April 1971.) £5; \$14.50.

WHEN the leaves of a plant are exposed to the elements, the First Law of Thermodynamics is satisfied when the net gain of heat from radiation and metabolism is balanced by heat losses in the form of convection and transpiration. The temperature of a leaf needed to achieve this balance is seldom identical with the temperature of the surrounding air. Moreover, the difference of temperature between the leaf and the air is usually greatest at the centre of the lamina and least around the periphery. This lack of uniformity coupled with the thinness of most leaves makes mean leaf temperatures difficult to measure by conventional thermometry. Ecologists are therefore interested in ways of inferring the temperature of a leaf from a knowledge of its heat balance and David Gates has been the main exponent of this approach for the past decade. The *Atlas of Energy Budgets of Plant Leaves* which he has now produced in collaboration with LaVerne Papian ex-

ploits the ideas and measurements which he and his colleagues have described in a long series of papers.

An introduction of eighteen pages provides the theoretical and experimental basis for the tables and graphs which occupy most of the book. There are 280 tables each giving an estimate of leaf temperature and transpiration rate for selected values of air temperature, absorbed radiation, windspeed, humidity, leaf dimensions and stomatal diffusion resistance. The computer program used to obtain these tables was developed to plot 110 graphs showing "two-dimensional slices through the multi-dimensional space" which the heat balance of a leaf occupies and salient features of these graphs are discussed briefly. Few workers would have tackled such a formidable exercise and probably nobody but Professor Gates could have persuaded the Academic Press to accept the risk of publishing research material in such an unusual form. Unfortunately the book suffers from two major defects: the physical and biological bases of the calculations are unsound; and many workers will find it easier or more accurate to measure leaf temperatures or to calculate them from first principles than to use a complicated set of tables and graphs involving simultaneous interpolation of five or six variables.

The physical basis of the work can be criticized on four grounds. First, there is no reference to the existence of temperature differences across leaf surfaces, which may exceed 10°C for a large leaf exposed to strong radiation in a light wind. Indeed, it is not clear what average temperature was used to determine the heat transfer coefficients used in all the calculations (and quoted twice without units). Second, forced convection coefficients proportional to the square root of the windspeed have been used in conditions where free convection must be important, for example, when a leaf $5 \times 10\text{ cm}$ and 40°C hotter than the surrounding air is exposed to a windspeed of only 10 cm^{-1} . Third, sensible and latent heat transfers are treated as unrelated processes calculated from coefficients which change inconsistently with wind speed and leaf dimensions. Because the mechanism of heat transfer across a boundary layer is similar to the transfer of mass, the ratio of the two transfer coefficients is usually taken as a constant which can be calculated from the molecular diffusion coefficients. Fourth, many workers will object to the unqualified extrapolation of wind tunnel measurements to field conditions because the effect of turbulence and leaf flutter on transfer processes is not yet fully understood.

The main biological defect in the book is the assumption that the stomatal diffusion resistance for all leaves can

be expressed as a single term r_i irrespective of whether the leaves have stomata on one or both epidermises. Ecologists looking at the tables and graphs may not immediately realize that they all refer to amphistomatous leaves with the same resistance $2r_i$ for each epidermis. The fact that r_i refers to the sum of two parallel resistances is never mentioned. The calculation cannot be applied to hypostomatous leaves because the ratio of sensible to latent heat transfer is different for the abaxial and adaxial surfaces.

The presentation betrays signs of lack of coordination between the authors. The equation on which all the calculations are based contains a misplaced bracket and a symbol for the latent heat of evaporation in the denominator instead of the numerator of a term. In this equation, D is the dimension of the leaf in the direction of the wind and W is the crosswind dimension, but a few pages later these symbols are interchanged in a diagram and the crosswind dimension becomes L in the text. Units are very confused: heat flux is $\text{cal cm}^{-2}\text{ min}^{-1}$ in the introduction, $\text{ERGS/CM}^2/\text{SEC}$ in the tables, and both are used in the graphs; diffusion resistance is min cm^{-1} in the introduction and sec cm^{-1} in the tables linked by the statement that min cm^{-1} is "simply $\text{sec cm}^{-1}/60\text{ sec min}^{-1}$ ".

Even if all the approximations and assumptions are accepted, it is difficult to see how the book would be used to estimate the temperature and transpiration rate of a leaf in a given environment because the chances are so heavily against the specification of an environment conforming to the conditions assumed for a particular table or graph. To make the exercise even more difficult it is necessary to specify the "absorbed radiation", that is, the net radiation absorbed by the leaf less its long wave emission. Unlike the net radiation flux, this quantity cannot be measured directly by any instrument which has yet been devised.

The authors claim in their preface that the book is "intended for use by botanists, ecologists, foresters, agronomists, hydrologists and others interested in the exact quantitative response of plants to environment". It is misleading to suggest that any of the calculations in the book are exact: they are all approximations. In the context of a research paper read at a meeting or even publication in a journal, approximations are acceptable for the sake of discussion and illustration, but publishing the same material in a book without reference to uncertainties or errors does the subject a disservice. Ecologists and others who try to get their bearings from this atlas are likely to find that they have set off in the wrong direction.

J. L. MONTEITH

Cancer Treatment

Single Agents in Cancer Chemotherapy. By Robert B. Livingston and Stephen K. Carter. Pp. x+405. (IFI/Paleum: New York and London, 1970.) \$20.

THIS book brings together and summarizes the results of clinical trials conducted since 1955 by the National Cancer Institute of the United States on the effects of certain anti-cancer agents given to patients with a range of specified types of malignant disease. As the title implies, the data all concern the effects of the agents given alone rather than in combination with other drugs or with other forms of therapy. The information is presented in tabular form and is arranged for each drug according to type of disease, and numbers and percentages of patients responding to stated dose schedules. Sixteen drugs have entire chapters devoted to them. These are nitrogen mustard (HN2), cyclophosphamide, chlorambucil, melphalan, busulfan, methotrexate, 6-mercaptopurine, 5-fluorouracil, cytosine arabinoside, hydroxyurea, actinomycin D, mithramycin, vinblastine, vincristine, procarbazine and prednisone (and prednisolone). Data from trials on another nine agents are considered more briefly.

Only highly selected data have been summarized. For inclusion, reports had to fulfil certain medical and scientific criteria and, with few exceptions, to be published in the English language.

The principal value of the book lies in the brief comments on the role in cancer therapy of each of the agents reviewed. Experienced chemotherapists may not agree with some of the conclusions—for example, the overall response rate (22 per cent) of patients with melanoma to dimethyl triazeno imidazole carboxamide compares favourably with that to any other agent used against this form of malignant disease. Nevertheless, such statements provide a most useful background against which to judge the effects of new agents and new regimens.

The book holds little to interest those not working directly in the field of cancer chemotherapy, although reading it might engender in the mind of the non-specialist the thought that, if there existed for the more common types of cancer more effective anti-cancer agents, the need for painstaking comparisons of only weakly effective treatments might largely disappear.

FRANCIS J. C. ROE

Lipid Handbook

Lipid Metabolism. Edited by S. J. Wakil. Pp. xi+613. (Academic: New York and London, January 1971.) \$28.50; £13.30.

THIS book contains a collection of contributions on major topics in the field of lipid biochemistry by contributors

who, with the sad exception of the late Professor G. Hübscher, are all actively engaged in the forefront of their respective fields of research. The editor aims to provide a comprehensive account of major achievements and trends in the field for investigators, teachers and students. The need for this undoubtedly exists, for it is more than a decade since the last comprehensive reference book on lipid metabolism was published (*Lipide Metabolism*, edited by K. Bloch, J. Wiley and Sons, New York, 1960). In an area of biochemistry where there has been formidable growth, ten years is far too long.

The titles of the chapters suggest a textbook thoroughness in their selection, which is not altogether typical of their contents. As one would expect, there are chapters on the metabolism of fatty acids, sterols, glycerides and phospholipids, as well as more specialized contributions on bacterial lipids, on prostaglandin metabolism and on the biosynthesis of aromatic amines and of polyisoprenoid quinones. The contributions themselves suggest that the writers had considerable freedom in their choice of presentation. Several contributors have chosen to discuss their own interests at length, treating other topics much more concisely. Thus, in his chapter on fatty acid metabolism, Wakil gives an extensive account of the enzymology of fatty acid synthesis and the numerous possibilities of its control, but only briefly describes the enzymology of fatty acid oxidation. Inevitably, this kind of presentation is rather unbalanced, but to my mind the disadvantages are usually redressed by the interest of the more detailed sections. Textbooks are rarely stimulating. Other contributors have used a more balanced approach. Hübscher, for instance, has written an exemplary review on glyceride metabolism which will undoubtedly be of great use to students, teachers and research workers.

In a single volume covering such a large field there will inevitably be some topics which are left out. In the chapters dealing with the metabolism of lipids with cyclic structures, and in the chapter on prostaglandins, the emphasis is strongly on the mechanisms of reactions: it is necessary to look elsewhere for information about regulatory phenomena and about the physiological function of some of the compounds discussed. It seems a pity that Samuelsson decided to omit even the briefest discussion on the biological functions of prostaglandins. In this respect, fatty acid metabolism is better served by Wakil and Bressler and glyceride metabolism by Hübscher. All three contributors provide a broader biological perspective for their subjects. I felt that another omission was a chapter on the role of lipids in membranes, in view of the current interest in this topic.

Lennarz makes some interesting comments on the subject, principally with regard to bacterial membranes, but that is all. Doubtless Wakil will remedy this omission if his promised second volume comes into being.

In summary, *Lipid Metabolism*, covering the literature to the end of 1969, is a much-needed book. That it does not cover all aspects of lipid biochemistry is not surprising in view of its size. In any case the amount of information present makes the book an essential acquisition for anyone interested in the field. Much of the contents is stimulating and challenging. I found the phospholipid chapter particularly so. Towards the end of the book the reader may find himself hastening to look up the definition of a lipid. He may then wonder at the elegant and precise approaches that have been used to study such an ill-defined group of compounds.

JILL ILIFFE

Immune Machinery

Immune Surveillance: Perspectives in Immunology. Edited by Richard T. Smith and Maurice Landy. Pp. xvi+536. (Academic: New York and London, March 1971.) £5.60.

IN each of the past three years, a conference, under the sponsorship of the National Institute of Allergy and Infectious Diseases, has been held at Brook Lodge, Augusta, Michigan. It is in fact a sort of immunological masters' tournament—the entry is restricted to those who have done well in previous years and the few promising youngsters whose performance on the circuit augurs well for their future. A few distinguished but less active seniors are asked along for the occasional comment. All that is lacking is the excited audience and the hot-dog sellers.

The book which is the subject of this review provides the link between the conference and a wider public, but unfortunately it lacks the dramatic impact of the occasion itself; to continue the golfing analogy, it is akin to an edited television recording. I would also dispute the claim that the conference was in any sense an informal occasion. The very fact that a record was made—as far as can be judged very efficiently by stenotypy—in the presence of a distinguished group of scientists effectively precludes informality. Nevertheless there must be no doubt that this book records an important occasion in the world of immunology.

The most significant item is the statement and discussion of Jerne's new theory on the generation of antibody diversity and self-tolerance. The editors have skilfully summarized the notions presented in the form of a flow sheet, and indeed throughout the book their inter-

pretative comments are most helpful. The contributors have also occasionally been permitted useful afterthoughts.

Contemporary notions of receptor theory and recognition mechanisms are thoroughly aired and the concept of immunological surveillance (and its failure) in relation to tumour growth is discussed in a singularly clear manner in the session chaired by R. S. Schwartz and introduced by K. E. Hellström. The discussions are revealed as lively and one is grateful for the fact that several times participants are recorded as unable to understand the points at issue. There is a mildly self-congratulatory air about the meeting but this is fitting and one must agree with Chairman Smith (p. 47) that the affair was an intellectual *tour de force*.

The book is suitable for purchase by the libraries of all interested institutes and for the bedside of dedicated individuals. It is well laid out with good summaries of session topics in a manner engagingly similar to that adopted at the beginning of chapters in Victorian novelettes. There is no index or references.

A. J. S. DAVIES

Soil and Water

Soil and Water: Physical Principles and Processes. By Daniel Hillel. (Physiological Ecology: a Series of Monographs and Treatises.) Pp. xiv+288. (Academic: New York and London, January 1971.) \$14; £6.55.

THIS presentation of the fundamental physical principles governing the soil water system is intended for students of soil physics and for workers in a variety of disciplines for whom soil water is important. Part I covers the basic principles of soil and water, the physics of the state of water in the soil and its flow in saturated and unsaturated systems. Part II discusses the field water cycle, covering infiltration, redistribution of water in the soil profile, ground water drainage, evaporation, water removal by plants and the effect of energy balance on water balance. Appendices by C. R. Amerman and E. E. Miller describe methods of solving the flow equation. Over 400 references are collected at the end.

Hillel's treatment of this complex subject is didactic and clear, assisted by a number of schematic diagrams, but it is rather condensed so that there is little space for critical discussion. Brevity occasionally obscures comprehension, and the diagram on p. 76 is misleading in suggesting that a hanging water column is the normal way of applying 1 bar suction to a tension plate, but fortunately these lapses are rare.

The approach is thermodynamic: potential is the vital statistic of soil water. Hillel recognizes that a newcomer to the field may be confused by

the variety of units and symbols by which potential is expressed (five different pressure units occur in the book) but he missed the opportunity to give a lead in rationalizing these and relating them to SI units. The use of the term "wetness" for water content seems inappropriate, for it is not conventional and its subjective associations relate more to potential than to water content. Arbitrary soil water "constants" such as field capacity and wilting point are shown to have little physical significance, but it is a pity that in presumably abandoning the useful concept of available water capacity, no guidance is given on a more acceptable measure of the storage capacity of a soil profile.

These are relatively minor criticisms, and on the whole the book succeeds in its aim and should prove to be a valuable introductory text. It covers a wider subject matter and is more readable than E. C. Childs's book on soil water phenomena, but within its limits the latter both explains the physics better and deals with problem solving in more detail.

B. W. BACHE

Solids in the Infrared

Far-Infrared Properties of Solids. Edited by S. S. Mitra and S. Nudelman. (Proceedings of a NATO Advanced Study Institute, held in Delft, Netherlands, August 5-23, 1968.) (Optical Physics and Engineering.) Pp. viii+605. (Plenum: New York and London, 1970.) \$25.

THIS book presents the lectures given at a summer school in 1968 and is for postgraduate students of the solid state. Techniques of far infrared spectrometry (interferometric) are covered as are some particular aspects of the techniques of Raman spectroscopy which are relevant to very low frequency excitations. The most useful part of the book, however, is probably the middle section where in four chapters (out of nineteen) the main areas of solid state physics in which far infrared spectroscopy has been fruitful—magneto-optical effects due to free carriers in semiconductors, spin-wave and other excitations in magnetic solids, lattice vibrations in ionic crystals which order ferroelectrically, electronic excitations in superconductors—are reviewed. The other chapters take up more specialized topics and the treatment varies considerably.

D. H. MARTIN

Cardiology

Auscultation of the Heart and Phonocardiography. By Aubrey Leatham. Pp. 151. (J. and A. Churchill: London, 1970.) £4.00.

THIS excellent monograph is a comprehensive account of auscultation of the heart and is a record of the author's

extensive researches into this subject. The basic principles of auscultation and phonocardiography are dealt with in the first chapter and subsequent ones are concerned with the sound events in the cardiac cycle in health and disease. Various related problems are critically discussed and well illustrated by photographically recorded phonocardiograms. This book can be recommended for medical students and postgraduates but it is clear that the author assumes that the reader has considerable knowledge of cardiology. Leatham's book may be considered an important contribution to the literature on cardiovascular disease. The printing, illustrations and paper are of the highest order, references adequate although relatively few and it is recommended as an authoritative dissertation on this subject. The index, however, is short for a reference book with so much factual information.

W. BRIGDEN

Quaternary Quarterly

Quaternary Research: an Interdisciplinary Journal. Edited by A. L. Washburn and Joe S. Creager. Vol. 1, No. 1: Pp. 132. (Academic: New York and London, September 1970.) \$10.00 each volume.

THIS is the first number of a new periodical, to be issued four times a year, and costing \$30 per year for institutions and \$10 for personal subscribers. The subject of quaternary research is wide, covering all aspects of environmental history in the past two million years or so. A number of periodicals already cater for the same field, and I doubt whether there is a further need for yet another journal. In such an interdisciplinary subject as quaternary research, there are advantages in bringing together different facets of the same subject under the cover of one journal; advances on the subject can be kept under surveillance and they are more readily accessible to the reader than if they were dispersed in the journals of parent disciplines. On the other hand, the concentration into such an interdisciplinary journal may result in the separation of quaternary disciplines from their parent subjects, which is to be regretted because it is important that the relevance of quaternary research to these parent subjects should remain in front of readers of journals devoted to those parent subjects. The first issue of the journal contains articles on a wide variety of subjects, from quaternary meteorology to stratigraphy, thermokarst and tephrochronology. Some of the articles are reviews, which could be useful to quaternary researchers—certainly a better service than provided by over specialized articles. If a high standard can be maintained, the journal will certainly be most welcome.

R. G. WEST

CORRESPONDENCE

Smoking and Cancer

SIR,—I was pleased to read your editorial "Premature Puff for Smoking Beagles"¹ and add more information on Dr Hammond's and the American Cancer Society's attitude toward science, methodology, and his colleagues.

Washington University undertook a project early this year, under my direction, with the aim to review some of the crucial data linking smoking to disease. Ten distinguished men and women scientists, from as many leading universities and laboratories, agreed to provide authoritative guidance and to advise us on how to set up conditions that would assure a fair, unbiased and authoritative review of the data. An invitation was also extended to Dr Cuyler Hammond, of the American Cancer Society, to meet with this panel. He was given assurances of all possible safeguards in the use of his data. Dr Hammond's reply was a flat refusal.

Ever since his first survey of smokers and nonsmokers in 1952, the methods by which Dr Hammond obtained data and analysed them have been thoughtfully criticized by some of the world's outstanding statisticians and scientists. The problems created by the objections were never adequately dealt with. Yet there are disturbing possibilities that the association between smoking and lung cancer, presented with such conviction by Dr Hammond, is a spurious by-product of biased sampling methods. There is an equally disturbing possibility that much of the relationship between smoking and lung cancer in Hammond's data may actually be an expression of occupational exposures hidden within these data and not brought out by adequate analysis. As Dr Hammond continues to produce publication after publication based on these same data, many anomalies of the population studied become apparent. In few measures or observations does this study population resemble the make-up of the population of this country. It is becoming increasingly puzzling who really is represented by that sample collected by volunteers of the American Cancer Society.

Most disturbing, however, are some of the ambiguous impressions created by Dr Hammond. Very often these occur in his testimony to Congress, or in some instances his conclusions are based on apparently distorted data summaries. Such testimony does not go through any screening process at all, yet it has an immense influence on planned or actual legislation.

This series of events has serious

implications for science in a free society, particularly for the fuzzy area where science affects public health matters. The refusal of these investigators to make their data available for public review threatens the basic tenet of science in a free society. As often before, the question arises if science can exist unless its actions are kept public. Unfortunately, and again, as often before, the question of credibility of claims based on secret data arises over an extremely unpopular issue.

It is obvious that we cannot, as a community of scientists, examine in detail the data collected by each individual member. It is also equally clear that testimony of experts very often must be accepted. However, as a practical procedure, published results and testimony have meaning only because we assume that, in the event the need arises, the actual data on which the investigator or the expert bases his conclusions are open to inspection. Otherwise, the claims of investigators or the testimony of experts completely lose their credence. Perhaps the matter was stated most succinctly by Bertrand Russell in his discussion of the limitations of the scientific method: "... it is clearly impossible that each of us should verify the facts of geography; but it is important that the opportunity for verification should exist, and that its occasional necessity should be recognized." The transactions of the scientific community must be conducted in public. This tenet is deeply engrained in the process of scientific inquiry. I quote, for instance, the American Association for the Advancement of Science's Committee on Science in the Promotion of Human Welfare: "Science gets at the truth by a continuous process of self-examination which remedies omissions and corrects errors. This process requires free disclosure of results, general dissemination of findings, interpretations, conclusions, and widespread verification and criticism of results and conclusions" (*American Scientist*, 53, June 1965). Data on which scientific claims are based must be public in a sense that they are available for review. Conversely, can one give credence to any widely disseminated claims based on observations which are kept secret or confined? This question is especially pressing in instances where long-range research plans and public actions affecting many individuals have to be based on scientific inference. To give credence to reports based on privileged data is to destroy the validity of the scientific method. The access to data has been restricted before, usually in a "popular" cause. But

whether the issue is one of attacking unpopular genetic theories, or of the right to make a profit by curing cancer, or in the guise of protecting innocent children from a dreadful smoking habit, censorship and secrecy are anathema to science. By his ill-advised actions, Dr Hammond has impugned the credibility of his own claims. There is no review board to which such matters can be referred except the integrity of individual scientists. Thus, I can do no more than bring this matter to the attention of my colleagues. The final action is up to the consensual process of the scientific community.

Yours faithfully,

THEODOR D. STERLING

*Department of Applied Mathematics
and Computer Science,
School of Engineering and
Applied Science,
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¹ *Nature*, 230, 547 (1971).

Indian Brain Drain

SIR,—A few comments on Mr Parthasarathi's article entitled "Brain Drain from Developing Countries", which appeared in the March 12 issue of *Nature*. He has made several good points on this problem. However, in his section on Joint Initiatives, he has suggested that American universities should counsel Indian students to cater to Indian needs. This scheme sounds fair if the Indian government sponsored the graduate students' education. In most cases, the financial assistance to the graduate students is provided by the American universities—so is it not unrealistic to expect American counsellors to be over-enthusiastic in providing the "home country interface"?

Yours faithfully,

P. K. GOVIND

*National Center for
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Problematic Chromosomal RNA

SIR,—The comment by your molecular biology correspondent¹ concerning nuclear and, in particular, chromosomal RNA requires comment. We must assume that his interests lie elsewhere or that, perhaps, he has been on an extended

vacation, so unacquainted is he with the literature on chromosomal RNA. If this were not so he would have immediately appreciated that Heyden and Zachau (*Biochim. Biophys. Acta*, **232**, 651; 1971) failed to recover chromosomal RNA from calf thymus chromatin because they used no procedure which would have made it possible. Thus, chromosomal RNA is covalently bound to protein and the latter must be removed before the RNA can be recovered by phenol extraction. The pronase used by Heyden and Zachau, unable to digest even its own RNAase impurity, was clearly incapable of digesting the chromosomal RNA protein. In addition, the DNA-RNA hybridization conditions of Heyden and Zachau were unsuitable (very much too low product of RNA $\times$ time) to reveal the hybridization of chromosomal RNA, even had they recovered it.

The over-interpretation, based on lack of knowledge of the field, which your molecular biology correspondent gives to Heyden and Zachau's article is matched of course by that which another of your correspondents has given to the findings of Clark and Felsenfeld (*Nature*, **229**, 230; 1971) concerning the structure of chromatin. One would hope that your correspondent would wish to conceal his lack of knowledge of and insight into nuclear matters instead of openly and publicly wallowing in it.

Yours faithfully,

JAMES BONNER

California Institute of Technology,
Pasadena,
California 91109

¹ *Nature*, **231**, 18 (1971).

Our molecular biology correspondent writes:

Attack may be the best form of defence, and I am happy to see that Professor Bonner has lost none of his legendary agility. His attempt to muddy the water by impugning the activity of Heyden and Zachau's pronase, however, savours of desperation; as well suggest that their

spectrophotometer is playing them false and all their RNA concentrations are wrong. Were the destruction of contaminating nucleases in relation to the autolysis rate of the protease as simple an issue as Professor Bonner makes out, less sweat would have been expended on the various chromatographic methods of purification that are to be found in the literature. The professor chooses, of course, to ignore that Heyden and Zachau took care to assay the activity of their pronase. However, the message implicit in his letter, on the dangers of overpurifying one's enzymes (at least if it is low-molecular weight RNA that one is after), will no doubt be widely appreciated. As to the hybridization conditions, I would merely direct Professor Bonner's attention to a paper by Sivopal and Bonner (*Proc. US Nat. Acad. Sci.*, **68**, 387; 1971), which will make it clear that Heyden and Zachau used both a higher RNA concentration and a longer time.

I am sorry that Professor Bonner has not divulged the nature of his complaint with my fellow Grub Street hack, who commented without his permission on the paper of Clark and Felsenfeld. We scribes of course have learned to bear with fortitude the periodic outbursts of scorn and abuse from splenetic *savants*, but it seems churlish to try to deny us even a little public wallowing.

British Diary

Monday, June 28

People and Roads: The Social Impact of Urban Transport Policies (8 p.m. discussion) Mr Peter Hill and Mr John O'Malley, British Society for Social Responsibility in Science, at the Museum Tavern, Great Russell Street, London WC1.

Thursday, July 1

Fellows' Open Day (from 10.15 a.m.) National Institute of Agricultural Botany, Huntingdon Road, Cambridge. AGM, Friday, 2.15 p.m.

Pancreatitis (5.15 p.m.) Mr J. Trapnell, University of London, at Westminster Medical School, 17 Horseferry Road, London SW1.

Friday, July 2

Intervertebral Disc (10 a.m. symposium) Bone and Tooth Society, at Princess Margaret Rose Orthopaedic Hospital, Fairmilehead, Edinburgh.

The Blood Supply of Skin Grafts (11 a.m.) Mr T. J. S. Patterson, University of London, at the Royal Postgraduate Medical School, Hammersmith Hospital, Du Cane Road, London W12.

Reports and Publications

not included in the Monthly Books Supplement

Great Britain

Advisory Committee on Gas Council Researches—Report 1968/69. Pp. 12. (Leeds: The University, 1970.) [264]

Education and Science in 1970—a Report of the Department of Education and Science. Pp. 96. (London: HMSO, 1971.) 60p net. [264]

World Development Handbook. By Juliet Clifford and Gavin Osmond. Pp. xi+172. (London: Charles Knight and Co. Ltd, 1971. Published for Overseas Development Institute Ltd.) £1.50. [264]

University of Oxford. Annual Reports, 1969–1970. Pp. 28. (Supplement No. 6 to the *University Gazette*, Vol. ci, April 1971.) (Oxford: The University, 1971.) 40p. [264]

The Marine Fauna of the Cullercoats District. 5: Arthropoda 3c—Crustacea; Copepoda. By Jo. Boasanyi and H. O. Bull. (Report of the Dove Marine Laboratory—Third Series, No. 17.) Pp. 59. (North Shields: The University of Newcastle upon Tyne, Dove Marine Laboratory, 1971.) 37p. [264]

The Control of Bovine Mastitis. Edited by F. H. Dodd and E. R. Jackson. (Papers given at a meeting organized by the British Cattle Veterinary Association and the Agricultural Development Association, held at Reading University, January 5–6, 1971.) Pp. 130. (York: The Agricultural Development Association, Cliftonfield, Shipton Road, 1971.) £1.40. [284]

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